PB82-136367

NPDES Compliance Monitoring Inspector Training: Biomonitoring

(U.S.) Environmental Protection Agency
Washington, DC

Jun 81

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
		PB82 136367

4. TITLE AND SUBTITLE	5. REPORT DATE (Date of Publication)
NPDES Compliance Monitoring Inspector Training Biomonitoring	6/81
	6. PERFORMING ORGANIZATION CODE 344005
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Enforcement Division (EN-338) Office of Water Enforcement and Permits Environmental Protection Agency 401 M Street S.W., Washington, D.C. 20460	10. PROGRAM ELEMENT NO. 2BG647 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Office of Water Enforcement and Permits Environmental Protection Agency 401 M Street S.W., Washington, D.C. 20460	13. TYPE OF REPORT AND PERIOD COVERED Technical Training 14. SPONSORING AGENCY CODE 700/02
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

This document is part of a set of five documents which make up the National Pollutant Discharge Elimination System (NPDES) Compliance Monitoring Training Program. The training modules were developed by the Environmental Protection Agency (EPA), Office of Water Enforcement and Permits (OWEP), to instruct NPDES inspectors in various aspects of conducting NPDES Compliance Monitoring Inspections. The five training modules in this set are: 1) Overview, 2) Legal Issues, 3) Biomonitoring, 4) Sampling Procedures, and 5) Laboratory Analysis.

The modules were designed to be used as a self-taught course or as the basis for a lecture course to supplement on-the-job training for NPDES Inspectors. The modules should be presented by experienced and knowledgeable staff who can answer any questions, discuss policies regarding the topic being presented, and conduct on-the-job training. Each module covers a specific aspect of a compliance inspection. Instructors for a particular module may be selected according to their expertise, and training sessions could be scheduled based on the needs, the resources, and the time available to the Region. The modules can be presented individually or as a complete package.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Monitoring Water Pollution Wastewater Control NPDES Inspector Training Compliance Monitoring	Compliance Monitoring 1) Overview 2) Legal Issues 3) Biomonitoring 4) Sampling Procedures 5) Laboratory Analysis	

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to the public	Unclassified	94
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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Washington, DC 20460

PB82-136367

EPA

NPDES Compliance Monitoring Inspector Training

Biomonitoring

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

NPDES COMPLIANCE MONITORING

INSPECTOR TRAINING MODULE

BIOMONITORING

U.S. ENVIRONMENTAL PROTECTION AGENCY

ENFORCEMENT DIVISION

OFFICE OF WATER ENFORCEMENT AND PERMITS

COMPLIANCE BRANCH

JUNE 1980

DISCLAIMER

This module has been reviewed by the Office of Water Enforcement and Permits, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ACKNOWLEDGEMENT

These modules were developed by Barbara A. Schick, Claire M. Gesalman, Duane Geuder, Edward Bender, and with contributions by Dave Shedroff, all of whom are staff members of the Enforcement Division, Office of Water Enforcement and Permits. The Compliance Branch, Enforcement Division, Office of Water Enforcement and Permits, wish to express their appreciation to the secretarial staff for the assistance provided in the preparation of this module, especially Mrs. Mary F. Rogers and Mrs. Wilma Haney.

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FOREWORD

The National Pollutant Discharge Elimination System (NPDES) Compliance Monitoring Inspector Training Modules were developed by the Environmental Protection Agency (EPA), Office of Water Enforcement and Permits (OWEP), to instruct NPDES inspectors in various aspects of conducting NPDES Compliance Monitoring Inspections.

The EPA Regions have identified a need for training materials to instruct new employees in conducting NPDES inspections. Training seminars that are currently offered either do not address the training needs of an NPDES inspector or are not available due to limited resources or conflicting course schedules. These training modules were developed to fill the Regions' need for in-house inspector training.

The objectives of the training modules are:

1. To acquaint new inspectors with the NPDES Compliance Inspection program;

2. To serve as a refresher course for experienced NPDES Inspectors;

3. To assure consistency in the NPDES Compliance Inspection program; and

4. To inform and instruct inspectors concerning new inspection procedures.

The modules were designed to be used as a self-taught course or as the basis for a lecture course to supplement on-the-job training. The modules should be presented by experienced and knowledgeable Regional staff who can answer any questions, discuss Regional policies regarding the topic being presented, and conduct on-the-job training.

The module format was chosen for this training program because of its flexibility. Each module covers a specific aspect of a compliance inspection. Instructors for a particular module may be selected according to their expertise, and training sessions could be scheduled based on the needs, the resources, and the time available to the Region. The modules can be presented individually or as a complete package.

An outline of information contained in the individual training modules is listed below. There are currently five NPDES Compliance Monitoring Inspector Training modules:

1. The Overview module gives the inspector an overview of the compliance program and a brief summary of the different types of compliance inspections.

FOREWORD (Continued)

2. The Legal Issues module outlines the legal issues which must be addressed during an inspection and legal information that will assist inspectors in performing their duties.

3. The Biomonitoring module outlines the principles of biomonitoring and the role of biological testing in the inspection program.

4. The Sampling module details the sampling procedures that an inspector uses when conducting a sampling inspection.

5. The Laboratory Procedures module outlines the procedures and information necessary for an inspector to perform an effective evaluation of a permittee laboratory.

The layout of the text of each module is on a half page so that students may include their notes with the text.

These training modules were developed for the Regions and are designed to be used by the Regions for in-house training. If these modules are to be a success, the Regions must participate in their ongoing development. This can be accomplished by providing EPA Headquarters with changes or information which Regional instructors or managers believe would improve the modules. The format of the modules can be updated and revised at OWEP as the need arises as they were developed and produced at EPA Headquarters. Cooperation and commitment to training by the Regions will promote the development of a useful training document.

These training modules were developed primarily for Regional NPDES Inspectors; but they are also available to other interested parties such as State offices, attorneys, other program offices, facility owners and operators, and members of the general public.

Comments, information, and suggestions to improve the modules should be addressed to the:

Technical Evaluation and Support Section (EN-338)
Office of Water Enforcement and Permits
U.S. Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460

Modules covering new topics may be added to the existing ones as the need arises. Subject suggestions for future modules should be sent to the above address.

Requests for training modules will be handled at the above address depending on available supplies.

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Effluent Biomonitoring

I. INTRODUCTION

Purpose of This Module

Reference: The Interim NPDES Compliance Biomonitoring Inspection Manual

Effluent Biomonitoring

As a result of this training, the trainee will be able to:

(1) Define the terms effluent biomonitoring, LC50 and EC50, and explain how they relate to the National Pollutant Discharge Elimination System (NPDES) permit program

(2) Describe the methods of determining the toxicity of pollutants through acute bioassays

(3) Describe the NPDES compliance biomonitoring inspection process

(4) Distinguish between a Compliance Biomonitoring Inspection, a Performance Audit Inspection, and a Compliance Evaluation Inspection (CBI, PAI, and CEI) for biomonitoring

(5) Determine how a CBI can best be used

I. Introduction

A. Scope

The purpose of this training module is to provide all inspectors with an understanding of the techniques and potential applications for Compliance Biomonitoring Inspections (CBIs). As a result of this training, the inspector should learn the purposes of the CBI and recognize some of the resources and technical requirements necessary to use a CBI. Our discussion will cover the background of effluent biomonitoring, definitions, and general procedures for conducting biomonitoring inspections. In addition, we will discuss choosing facilities for biomonitoring inspections,

interpreting the results of bioassays, and preparing the inspection report. The Interim NPDES Compliance Biomonitoring Inspection Manual MCD-62 (October 1979) should be used in conjunction with this training module as a reference for sample collection and toxicity testing procedures. For brevity this reference will be called the "Manual" in this module.

B. Definitions

Definitions

- biomonitoring
- acute toxicity testing

The term effluent biomonitoring or effluent biological monitoring, means measurements of the biological effects (e.g., toxicity, bioaccumulation, and biostimulation) of effluents on populations of indigenous organisms.

Acute toxicity tests (acute bioassays) are biological monitoring techniques which measure the lethal effects of a compound, mixtures of compounds, or effluents on an organism. We will discuss the use of acute bioassays to evaluate the toxicity of effluents. A glossary of these terms is provided in Handout 1.

Handout 1 - Glossary

C. Background

EPA Experience

EPA has used many different biological monitoring techniques to determine the effects of compounds and wastewater discharges on receiving streams. These techniques range from ambient (instream) monitoring of aquatic communities (e.g., species composition, indicator species, and community structure) to effluent monitoring (e.g., bioassays). Section 101(a)(3) of the Clean Water Act (CWA) states that it is a national goal to prohibit the discharge of toxic pollutants to our waterways in toxic amounts. Bioassays have been used to

develop effluent limitations and water quality criteria for some toxic chemicals, particularly under Section 307(a) of the CWA. However, toxicity data are available for only a limited number of compounds and in most cases, these data do not consider the interactions of compounds in the effluent which affect toxicity. No chemical test can detect toxicity; only living organisms can be used to determine toxicity.

Biomonitoring is an important part of EPA's strategy to control the discharge of toxic materials. Biomonitoring is used because no other technique can predict the biological effect of chemicals and changes in water quality. This strategy was developed from EPA's experience with toxicity testing and prompted by Sections 101, 301, and 307 of the CWA and recent judicial history.

The consent decree of June 7, 1976, between the Natural Resources Defense Council, Inc., and EPA (Natural Resources Defense Council et al. v. Train 8 E.R.C. 2120 (D.D.C. 1976)) required that the Administrator develop and promulgate regulations establishing effluent limitations and guidelines, new source performance standards and pretreatment standards for new and existing sources. Effluent limitations and new source performance standards require that Best Available Technology (BAT) economically achievable be considered in their development.

All three of the regulations must take into account the list of toxic pollutants categories given in Appendix A of the consent decree and commonly referred to as the list of 65 categories of toxic pollutants.

Handout 2: 21 Primary Industrial Categories

Handout 3: Compounds Considered to be Toxic

Handout 4: Priority Pollutants

The consent decree also required that these limitations be applied to 21 primary industrial categories (see Handout 2) named in the consent decree. In the course of developing the technical data base required to formulate limitations for the 65 categories of pollutants, EPA developed a list of specific compounds in ten categories which are considered to be toxic (Handout 3). This list (May 1977) included 129 compounds (Handout 4) which are known as priority pollutants.

The consent decree prompted the 1977 amendments to the CWA which outlined EPA's approach to control toxic pollutants. This approach included BAT quidelines and biological monitoring. BAT guidelines will be the most stringent level of treatment for any industrial category. However, effluents which meet BAT may still be toxic because the limitations have been developed based on available treatment technology without toxicity data for the effluents. Effluent guidelines for some industries may include limits for individual priority pollutants, however, the effluents from specific discharges may still contain other priority pollutants and other toxic compounds which are not limited by the permit. For some industrial categories, BAT may be equivalent to Best Practicable Technology (BPT) which is now used to issue permits. For these categories, wastewater treatment will not change. In addition, some effluent guidelines will not be promulgated for several years. Effluent biomonitoring can be used now to evaluate the toxicity of effluents and to assure that BAT mitigates the adverse biological effects of toxic effluents. The CBI uses acute toxicity tests to

identify effluents which are toxic. The alternative is to identify all compounds or mixtures of compounds in the effluent which are toxic, and to establish limits on each compound. This is impractical and much more costly than a direct screen of the effluent for toxicity.

Acute Toxicity Testing

D. Acute Toxicity Testing

Definitions:
-Effluent Biomonitoring

-LC50

Now it is necessary to define some other terms commonly associated with effluent biomonitoring (Handout 1). Acute toxicity tests are used to measure the effluent concentrations, expressed as a percent volume, that are lethal to or have some other adverse effect on 50% of the population of organisms within a prescribed period of time. If mortality is the effect being measured, the toxicant concentration is expressed as a median lethal concentration, or LC50. In other instances, mostly with invertebrates, death is not easily detectable, and indices such as immobilization or reduction of shell growth must be used to measure an adverse effect. The concentration of an effluent, expressed as a percent volume, that causes a defined adverse effect other than death in 50% of the test organisms within a prescribed exposure period is termed the median effective concentration, or EC50.

-Range-Finding Test

-QA Bioassay

-Definitive Test

Acute bioassays described in the Manual consist of static or flow-through tests performed as a 1) range-finding test, 2) a Quality Assurance (QA) bioassay, or 3) a definitive test. Range-finding tests are short term bioassays (8-24 hours) used to approximate

the range of the LC50 for a definitive test. Usually five organisms are tested in each concentration of the effluent with a range such as 50%, 10%, 1%, and 0.1% volume/volume dilution. The effluent concentrations used in the definitive test should include the LC50 concentration estimated from the preliminary range finding test. A QA bioassay with a standard toxicant (supplied by Environmental Monitoring and Support Laboratory (EMSL) -Cincinnati) is conducted to determine the sensitivity of the test organisms. The LC50 determined from the QA bioassay is compared to a standard range LC50 of the reference toxicant for the test organism. If the LC50 from the QA bioassay falls within the range, that batch of organisms can be used for testing. The definitive test consists of five or more effluent concentrations and a control with 20 organisms in each concentration. The definitive test is run for 24, 48, or 96 hours under static or flow-through conditions. This test provides an estimate of the effluent dilution that kills half the test population, the LC50. These procedures are described in more detail later in the module.

Handout 5: Clean Water Act of 1977

The acute toxicity test is the basis for the NPDES biomonitoring inspection. The biomonitoring inspection is authorized under Section 308(a) of the Clean Water Act of 1977 (Handout 5). This Section authorizes the Administrator of EPA to require the owner or operator of any source discharge to:

1. Establish and maintain records

2. Make reports

3. Install, use, and maintain monitoring equipment and methods (including, where appropriate, biological monitoring methods)

4. Sample the effluent

Advantages of Biomonitoring

E. Advantages of Biomonitoring

Biomonitoring has several advantages over chemical by chemical monitoring. Because the acute toxicity test uses living organisms which are indigenous to the receiving water, it provides an estimate of the potential effects of the effluent on the survival of that species in the receiving stream. Living organisms respond to the collective effects of all chemicals in the effluent. Therefore, acute toxicity tests measure the short term effects of compound interactions (i.e synergism and antagonism). The test measures the lethal effects of the effluent. Static bioassays of effluents can be run for as little as $400.00, while detailed chemical analysis can cost several thousand dollars. On-site 96-hour flow-through bioassays can be performed for about $5,000.00.

Limitations of Biomonitoring

F. Disadvantages of Biomonitoring

Effluent biomonitoring is not a panacea to solve all problems with toxic effluents. The test has several limitations which may require supporting chemical analysis or repeated testing to overcome. One limitation is that the composition of some effluents is highly variable. This problem also applies to sampling effluents for chemical analysis. The variability of the effluent can affect the results of a static bioassay, so

the sample should be collected as a 24-hour composite (described in the Manual) to minimize the variability. Flow-through bioassay techniques may be used to continuously monitor toxicity of an effluent. Another problem is that the acute toxicity test does not measure the persistence of the toxic effect; however, special procedures are included in the Manual to evaluate persistence. The primary complaint about effluent biomonitoring is that it does not identify the cause of toxicity, only the effect; but, as mentioned earlier, chemical analysis does not identify biological effects, it only identifies the chemicals present. Thus, if we wish to know the cause-effect relationship associated with an effluent, we must use chemical analysis to support biomonitoring. If the wastewater varies in composition over a period of time, it is best to perform chemical analysis (i.e., a Compliance Sampling Inspection (CSI)) concurrently with a CBI. If this is not possible, past CSI results should be reviewed and discussed in the inspection report. The persistence and acute toxicity of the effluent are used to extrapolate out results to establish safe concentrations of the effluent in the receiving stream (i.e., concentrations which are not chronically toxic). This procedure will be discussed later under Post-Inspection Activities.

Objectives of CBI

G. Objectives of CBI

As stated in the Manual, the objectives of the CBI are:

1. To serve as a screening mechanism, isolating toxic conditions in effluents which may not have been

detected through routine chemical analyses

2. To evaluate compliance with State water quality standards

3. To monitor toxics which may or may not be controlled through BCT (Best Conventional Technology) /BAT (Best Available Technology)

4. Evaluate permit limits

5. Develop enforcement cases

6. Investigate probable cause violations

7. Develop data for establishing permit limits

Inspectors Responsibilities

H. Inspectors Responsibilities

The inspector has certain obligations and responsibilities in conducting a biomonitoring inspection. These include:

1. Knowledge of biomonitoring permit conditions, effluent toxicity limitations, and related interim and final requirements set forth in the latest NPDES permit

2. Knowledge of EPA policies and procedures for conducting, interpreting, and reporting biomonitoring of wastewater effluents

3. Developing the inspection plan and scheduling the inspection

4. Conducting the on-site biomonitoring evaluation inspection

5. Preparing or assembling complete and accurate records of self-monitoring practices and other issues addressed during an evaluation inspection

6. Follow-up with the permittee and interested parties after the audit with regard to biomonitoring performance, quality control, and related compliance activities evaluated during the inspection

Phases of a CBI

I. Phases of a CBI

The CBI will be discussed in three phases: Phase I - Preinspection Planning, Phase II - Inspection Procedures, and Phase III - Post-Inspection Activities. You should refer to the Manual for detailed procedures for each phase. The description of these phases will help a new inspector understand biomonitoring.

II. PHASE I- PREINSPECTION PLANNING

II. <u>Phase I Preinspection Planning</u>

This phase includes both administrative procedures to select sites, notify permittees, and review files; and technical procedures to evaluate the sensitivity of test organisms and verify that equipment materials are ready for the inspection.

Criteria Used to Select Permittees for Biomonitoring

A. Selecting Permittees for a CBI

All inspectors should be aware of the criteria used to select permittees for biomonitoring inspections. CBI's

are frequently performed at facilities with major discharges of process water that are suspected to be toxic. This evidence may include inadequate self-monitoring reports, fish kills in the receiving stream, and citizen complaints which suggest that the effluent causes some health hazards. These are examples of probable cause.

CBI's may also be performed on a class of facilities defined by certain criteria in a neutral inspection scheme. For example, EPA Headquarters, a Region, or a State agency may suspect that a particular industrial category has a toxic effluent because the Effluent Guidelines Division, EPA, has found priority pollutants in the waste stream of some plants within this category. Therefore, CBI's could be performed on all major dischargers with a certain Standard Industrial Category (SIC) code. Ambient data (e.g., toxic hot spots) could be the basis to perform CBI's on all major discharges within a section of a stream. When classes of dischargers are inspected, individual permittees have been selected by a neutral scheme.

Equipment Requirements

B. Equipment Requirements

Equipment
a) types of labs
b) equipment
c) water
types
specifications

Effluent toxicity tests may be performed in either a mobile laboratory or a permanent facility. Permanent laboratory facilities are used primarily for static bioassays of effluents; mobile labs are usually equipped with dilutors to run flow-through tests. Permanent facilities are required to maintain cultures of test organisms. Depending upon the scope of the bioassay program and the equipment needed for testing, typical facilities include aquaria equipment, dilution systems, chemical-physical

monitors, and delivery systems. The source of the dilution water used in the tests may be ground-water, surface water, reconstituted water, or dechlorinated tap water. Holding, acclimation, and dilution water should all be temperature-controlled and aerated. Air used for aeration must be free of oil and fumes, and test facilities must be well ventilated. During the test, organisms should be isolated from any disturbances.

Construction Materials

Materials used to construct test equipment that will come into contact with the effluent or test organisms should be carefully chosen so that leaching, dissolution, or sorption are prevented. Glass, no. 316 stainless steel, and perfluorocarbon plastic should be used whenever possible. Plastics made without a plasticizer (for example, polyethylene, polypropylene, and fiberglass), can be used for holding and acclimating, in dilution water storage tanks, and in the water delivery system. Copper, galvanized material, rubber, brass, and lead should not be used. If materials such as vinyl garden hoses are used to pump effluents into the trailer, they should be rinsed thoroughly before testing and discarded after the test.

Dilutors

Flow-Through Dilutions

Design Considerations:
1) location
2) space
3) application
4) reliability
5) performance
6) cost

1. Dilutors

The flow-through, proportional-dilutor delivery system is the best system for routine effluent toxicity testing. The following design considerations should be used in planning your system:

a. Whether the apparatus will be installed and used in a permanent or mobile laboratory

b. Space and/or structural requirements for the delivery system, test chambers, effluent, and dilution-water storage

c. The applicability of the delivery system to specific effluent characteristics (high suspended solids, volatiles, etc.)

d. The dependability, durability, flexibility, and ease of maintenance and replacement of the system

e. The ability of the system to perform within acceptable flow rate and concentration limitations

f. The cost of the system

Operating Requirements for the Dilutor

The dilutor must provide for at least five complete water volume changes in 24 hours in each test chamber, plus sufficient flow to maintain an adequate concentration of dissolved oxygen ($\geq$ 4mg/l). The flow rates through the test chambers should not vary by more than 10% between chambers. The dilutor should be capable of maintaining the test concentrations in each test chamber within 5% of

the starting concentration throughout the test. Two of the most popular dilutor systems are: the solenoid valve and the vacuum siphon systems. Note: a solenoid valve system is available from Ace Glass, Inc., Vineland, New Jersey 08360 (609-692-3333).

Test Chambers

2. Test Chambers

Test chambers vary according to the type of test being conducted. For flow-through tests, test chambers should be constructed of 1/4-inch plate glass held together with small quantities of silicon adhesive. The size of the chambers may vary according to size of the test organisms and the facility, but the test solution should have a minimum depth of 5 centimeters in the chamber. All chambers should have either a glass or screen cover to prevent organisms from jumping out. Wide-mouthed, 3.9-liter soft-glass bottles are often used for test chambers in static tests.

Cleaning Equipment

3. Maintenance

All test chambers and any other equipment that comes in contact with the dilutor system must be washed to remove surface contaminants using the following procedures:

a. Soak and wash all equipment in a suitable detergent and water, preferably heated to a temperature of 50°C or greater. The detergent (powder or liquid) should be entirely synthetic (SPARKLEEN or ALCONOX).

b. Rinse with tap water (preferably heated to 50°C or greater).

c. Rinse with a fresh, dilute (5 percent) hydrochloric acid to remove metals and bases.

d. Rinse with tap water (preferably heated to 50°C or greater).

e. Rinse with acetone to remove organic compounds. When contaminated with a pesticide, test chambers must be rinsed with acetone before they are placed in the hot detergent to soak.

f. Rinse twice with dilution water.

It is important to clean all equipment thoroughly to avoid transferring toxicants from one experiment to another.

Test Organisms

4. Test Organisms

The test organisms to be used for toxicity testing depend on the salinity of the effluent. If you are testing a freshwater effluent, the

NOTES	LESSON BIOMONITORING
Handout 6: Recommended Test Organisms	fathead minnow (Pimephales promelas) and Daphnia (Daphnia magna) should be used. The species preferred for saltwater testing are the mysid shrimp (Mysidopsis or Neomysis) and the sheesphead minnow (Cyprinodon variegatus). Other species can be tested in conjunction with these species. The recommended fish and invertebrate species must be used for consistency and comparisons between sites (Handout 6). a. Age It is recommended that minnows be more than ten weeks, but less than ten months old and that invertebrates be in the juvenile stage of development. These are the most sensitive post-hatch life stages. If other fish are being tested, it is important that they be taken from the same year class; and the total length and weight of the fish should be approximately the same. b. Acclimation Disinfect holding chambers and other equipment with 0.5 percent commercial bleach for 1 hour. Brush thoroughly with disinfectant and rinse. Holding tanks should receive uncontaminated water of consistent quality at a flow rate of 2-5 tank volumes per day. c. Feeding Acclimation procedures are used to prevent stress on new

batches of test organisms caused by drastic environmental changes. During acclimation, environmental changes should be limited to a change of 3°C in water temperature or 3 ppt salinity in any 12-hour period, or a total change of 6°C or 6 ppt salinity. Over-crowding should also be avoided. The water should be aerated, if necessary, to maintain an adequate dissolved oxygen supply.

d. Disease Control
Test organisms should be fed at least once a day during acclimation and holding, and excess food and fecal material should be removed at least twice a week by siphoning. The organisms should be observed constantly, and a daily log of feeding schedules, behavior, and mortality should be maintained.

During holding, fish should be chemically treated to cure or prevent disease. However, if the fish are severely diseased, all should be discarded. Diseased invertebrates should also be discarded. Tanks that have held diseased organisms should be thoroughly disinfected with 0.5 percent commercial bleach before the tanks are used again.

Organisms from a permanent facility are transported to the test site in the water in which they were reared and

held. If the laboratory is mobile, the acclimation tank can be used for transporting organisms from the rearing and holding facilities to the test site. At the test site, dilution water (receiving water) is pumped to the laboratory for acclimating the organisms. If dilution water is not readily accessible to the laboratory, it can be transported to the laboratory and stored in a tank for use in the acclimation procedure and the toxicity tests. During transport, the organisms should not be subjected to any salinity or temperature changes.

At the test site, the organisms are acclimated to the test dilution water and temperature by gradually changing from 100% holding water to 100% dilution water over 24 hours. All organisms must be exposed to 100% dilution water and be held at the test temperature (+2°C) for at least 24 hours before tests are begun.

A group of organisms must not be used for a test if they appear to be diseased or otherwise stressed, or if more than 5% die during the 48 hours immediately preceding the test. If the organisms fail to meet these criteria, the entire group must be discarded and a new group obtained.

Dilution Water

5. Dilution Water

Dilution water is water to be used in preparing the different concentrations of the effluent. Dilution water is acceptable if healthy test organisms survive in it through the acclimation period and the toxicity test without showing signs of stress such as discoloration or abnormal behavior. The dilution water should be a sample of the receiving water and should be obtained from a point close to the outfall, but upstream and outside of the zone influenced by the effluent. It is preferable to pump the dilution water continuously to the acclimation tank and dilutor. However, it may be more practical to transport batches of water in tanks to the testing site as needed, and then continuously pump water to these systems from holding tanks.

Pretreatment of the dilution water should be limited to filtration through a nylon sieve that has 2-4 millimeter mesh to remove debris and/or break up large floating or suspended solids. The water should be obtained from the receiving water as close as possible to the time the test begins. It should not be obtained more than 96 hours prior to testing. If acceptable dilution water cannot be obtained from the receiving water, some other uncontaminated, well-aerated

surface or ground water, or commercial source may be used or prepared. This water must have a total hardness, total alkalinity, and specific conductance within 25% of the receiving water at the time of testing. The pH must be within 0.2 units of the receiving water at the time of testing.

All biomonitoring teams must have at least one experienced biologist who directs the preparation of equipment for the inspection. Minor equipment items and supplies should be duplicated to avoid unnecessary delays in cases of failure or breakage. Many Regions have developed checklists of equipment and supplies, such as Handout 7, to reduce problems with packing.

Handout 7: Equipment Checklist

Preinspection Coordination With the Permittee

C. Preinspection Coordination

Preinspection procedures also include a review of the permittee's compliance file, permit application, and previous inspection data to develop an understanding of the permittee's production process, wastewater composition and possible problems. If an on-site flow-through bioassay is planned, it is best to contact the permittee early. One way to reduce logistics problems is to supply the permittee with a list of questions before the inspection (Handout 8). In cases where the inspection is announced well in advance, it is important to compare the discharge characteristics during the inspection period with routine measurements (particularly for flow)

Handout 8: Preinspection Questions

to be sure the plant was operating normally. Variations of more than ten percent should be identified and explained in the inspection report.

The latest QA bioassay results should be reviewed before the inspection. The purpose of the QA bioassay is to determine the sensitivity of the test organisms to a reference toxicant, usually sodium lauryl sulfate. The QA bioassays may be performed during the inspection or as part of the laboratory routine. If the results are more than three weeks old, another QA bioassay should be conducted, either concurrently with the inspection or before the inspection.

III. PHASE II INSPECTION PROCEDURES

III. Phase II Inspection Procedures

Evaluation Inspections

A. Evaluation Inspections

The inspector will be conducting both sampling and nonsampling inspections related to biomonitoring. The nonsampling or evaluation inspection includes the PAI and the CEI. The procedures for these inspections are described in other training modules and specific manuals.

PAI

1. Performance Audit Inspections

Review Procedures

The PAI requires at least one inspector with experience in bioassays. The inspector must review the performance of the permittee's staff and evaluate their test and sampling procedures. The inspector should review all test and culturing procedures. Particular attention should be given to the following areas:

NOTES	LESSON BIOMONITORING
Condition of Test Organisms	a. What is the source, age, weight, and species of test organism? Do they appear to be healthy? At what temperature are they maintained? What and how are they fed? Are they isolated from test chambers and volatile components of the effluent? How are they treated for disease?
Test Procedures	b. How are test concentrations established? Are flow, temperature, D.O., etc. controlled or monitored during tests? What is the source of dilution water? Is there any chemical analysis to verify that the dilution water is a good control?
Recording Results	c. How are the test observations made and recorded? Is the data in a bound log? Are test conditions/ failures described? Do the log results correspond to DMR data?

Data Analysis

Sampling Procedures

d. What method is used to calculate LC50's?

Do the survival levels correspond to the requirements for a definite test?

e. When conducting the performance audit, the inspector must evaluate the permittee's effluent sampling practices and verify that:

Samples are taken at locations prescribed in permit

Sampling locations specified in the permit are adequate to provide a well-mixed and representative sample

The frequency of sampling is done in accordance with the permit

Grab sample devices are clean and properly operated

Sample containers are clean and appropriate

Automatic sample collectors are operating properly

Samples are properly preserved and shipped

Effluent samples for bioassays are used without preservatives or adulteration

There is no more than a 24-hour maximum holding time before sample is used for biomonitoring

Cross-contamination of samples is prevented

Testing equipment is routinely calibrated

Furthermore, a performance audit must be conducted for the permittee's laboratory. The inspector is responsible for evaluating facilities, test organisms, dilution water, test procedures, and test results.

CEI

2. Compliance Evaluation Inspections

The CEI is the simplest and least resource-intensive type of evaluation inspection. At facilities that conduct biomonitoring, a CEI consists of an examination of the permittee's self-biomonitoring files and records, analytical and bioassay laboratory, and production facilities along with the routine review of sampling records and reports.

Sampling CBI's

B. Sampling Inspections

Sampling inspections are an evaluation of an effluent based on sampling and testing. Sampling inspections can be conducted on-site or off-site. On-site biomonitoring sampling inspections include:

An 8- to 24-hour range-finding bioassay

A 96-hour flow-through bioassay

A 24-hour QA bioassay with a reference toxicant

Careful examination of the permittee's self-monitoring program

1. Effluent Sampling

When static bioassays are performed, samples of the effluent must be collected. Grab samples may be used if the composition of the effluent is very consistent (see Manual). However, composite samples should be used if the effluent is variable or retention time of the effluent in the treatment system is less than seven days. Composites should be collected for one full operating day. If the discharge is continuous, a 24-hour composite is required.

Sampling Procedures

Effluent samples should be taken at the point specified in the NPDES discharge permit. It is important that the sample represent the "normal or typical" discharge and operating conditions of the facility. Sampling should be based on an understanding of the short-term operations and schedules of the discharger. Samples should not be altered prior to testing, although they may be filtered through a

Handout 9: Effluent Sampling Procedures

2-4 millimeter screen made of stainless steel or Teflon. Handout 9 lists effluent sampling procedures.

Sampling Handling

Once the effluent grab sample has been collected, it must be stored in a covered but loosely sealed container. Violent agitation must be avoided, but gentle agitation may be necessary to disperse suspended solids before dilutions are made. Samples should be stored at 4°C in a refrigerator, a constant-temperature water bath, or an environmentally controlled room at the test temperature. The test should be conducted within 48 hours after collection.

Range-Finding Tests

Acute toxicity testing procedures include both range finding and definitive tests which were defined earlier. Although toxicity testing may be done under static or flow-through conditions, static tests are used primarily because they are less expensive and easier to perform.

2. Definitive Tests

The flow-through testing procedure is preferred for the definitive test because it eliminates concerns about holding time and wastewater variations. However, the flow-through is more expensive because it is done at the permittee's site. Therefore, it requires more planning and resources than

a static test performed in a permanent laboratory. In flow-through tests, the delivery system should be in operation for 24 hours prior to adding the test organisms; for static test, the effluent should be mixed well by stirring with a glass rod. The test conditions needed for determining an LC50 must include at least five concentrations and a control.

Criteria for Valid LC50

a. Three criteria must be met in order to calculate a reasonably accurate LC50:

   Concentrations must be at least 50% of the preceding effluent concentration.

   One concentration must have affected 65% of the organisms exposed to it, and one concentration, other than the control, must have affected less than 35% of the organisms. If 100% effluent does not affect more than 65% of the exposed organisms, it is then necessary to report the numbers that are affected. Toxicity must increase with higher effluent concentrations.

Handout 10: Definitive Test Requirements

b. Handout 10 lists the definitive test requirements. These are:

Conditions

   Twenty test organisms of a given species must be used for each concentration or

ten for each chamber (replicate test chambers).

If two or more species are to be used in the test chambers, they should be separated with Nitex screens.

Randomization of treatments is desirable (i.e., organisms should be assigned to concentrations based on statistical randomization techniques).

Loading must not exceed 5 grams of test organism per liter at temperatures of 20°C or less, or 2.5 grams/liter at temperatures above 20°C for flow-through tests.

Loading for static tests must not exceed 0.8 grams per liter at temperatures of 20°C or less, and 0.4 grams/liter at temperatures above 20°C.

Temperatures must be held to within $\pm$2.0°C of the acclimation temperature for both static and flow-through tests.

Dissolved oxygen concentration should not be permitted to fall below 40% saturation for warm water species and 60% for cold water species.

The test period begins when the organisms are first exposed to the effluent.

Organisms are not fed during testing, unless they are mature or newly hatched. The duration of the test

Test Results

Data Reporting

Handout 11: Data Sheet for Effluent Toxicity Test

is 24 hours for static tests and 96 hours for flow-through bioassays.

3. Test Results

Three types of results can be obtained from an acute toxicity test done on effluents:

Biological data

Chemical and physical data

LC50 calculations

a. These data are recorded on the data sheet shown in Handout 11. Biological data include:

Length and weights of representative test organisms, and age, where applicable

The number of affected (dead) organisms in each test container after each day of testing

b. Physical and chemical data include:

Dissolved oxygen, temperature, and pH measurements taken at the beginning of the test and each day thereafter in the control and in each effluent concentration

Specific conductivity, total alkalinity, hardness, and salinity measurements taken at the beginning of the test in

	the control and in each effluent concentration. Total ammonia nitrogen measured at the beginning and end of each static test in the control and in all effluent concentrations
Handout 12: Methods for Calculating LC50, EC-50	c. LC50 and EC50 calculations and their 95 percent confidence limits must be obtained for each set of data based on the initial volume percent of the effluent. Several statistical methods are available. Handout 12 discusses two of the more common methods: Litchfield and Wilcoxon, and log concentration versus percent survival. Finally, information that should be included in the report of the results is shown in Handout 13. Handout 14 provides a guide for day-to-day inspection activities.
Handout 13: Reporting Test Results Handout 14: Day-to-Day Activity Guide	
IV. POST-INSPECTION PROCEDURES	IV. <u>POST-INSPECTION PROCEDURE</u> Post-inspection activities described below include: Data evaluation Completing the Acute Toxicity Laboratory Evaluation Form (Handout 15) Completing the NPDES Compliance Inspection Report (Handout 16).
Handout 15: Acute Toxicity Laboratory Evaluation Form Handout 16: NPDES Compliance Inspection Report Form	

Data Evaluation

A. Data Evaluation

The primary purpose of a compliance biomonitoring bioassay is to establish compliance status with bioassay requirements in a permit. Bioassays can also be used to determine if a particular effluent is acutely toxic. Evaluation of biomonitoring data is based on the following parameters:

LC50 of the waste

In-stream waste concentration

Permit limits

Chemical parameters of the effluents associated with the bioassay, such as:

-Dissolved oxygen
-Temperature
-pH
-Conductivity
-Metals
-Organics

The LC50 can be determined by probit analysis or graphical technique as shown in Handout 12. The graphical technique does not provide confidence intervals for the LC50; therefore, statistical methods such as probit, moving average angle, or logit are preferred. The Manual provides a discussion of how to determine compliance using the LC50, the instream waste concentration, and an application factor. Since this is critical, it is reviewed here. Computer programs are available for the statistical methods from Mr. Charles Stephen, ERL-Duluth, MN (8-783-9510).

Data Interpretation

B. Data Interpretation

Bioassay results are evaluated by verifying that the test conditions (e.g., water quality, survival of controls, and loading rates) were acceptable and by estimating the impact of the effluent on the receiving stream. Continuous measurements of water quality in flow-through tests monitor changes in wastewater composition. Changes in the flow-rate during sampling may indicate that changes occurred in the manufacturing operation or stormwater runoff. Acute toxicity may be caused by water quality variation such as low dissolved oxygen concentrations (4.0 mg/l) or changes in pH. Water temperature and conductivity measurements can detect changes caused by different manufacturing operations. The biologist should examine the pattern of survival during the test. If the LC50 is the same at 24 hours as one estimated for 96 hours, the effluent probably contains toxic compounds which are degraded or volatile. In some cases, this toxicity could be reduced by increasing the retention time of the treated effluent. If the effluent causes deaths throughout the test period, toxic compounds in the effluent are probably bioaccumulated by the test organisms. The toxicity of the effluent is based on the LC50 and the instream waste concentration. The effluent may be highly toxic (LC50 1.0%), moderately toxic (1-25%), somewhat toxic (25-50%) or marginally toxic (50%). Highly toxic effluents require immediate action to reduce the toxicity. In most cases the permittee is probably violating permit limits already. Permittees with moderately toxic effluents should be considered for biomonitoring requirements in their permits. Permittees with somewhat toxic effluents and marginally toxic effluents should

be retested if they fail the IWC criterion.

The instream waste concentration (IWC) is the ratio of the effluent flow to the 7 day, 10 year low flow of the receiving stream. $IWC = \frac{Q\ effluent}{7Q10}$

(Note: In some cases, where 7Q10 is not available, the annual low flow value may be acceptable.)

The IWC is compared to the product of the LC50, expressed as the percent concentration of the effluent, and an Application Factor (AF). The Application Factor is intended to protect aquatic organisms from chronic toxicity outside the mixing zone. If the toxicity of the effluent is considered persistent (i.e., the effluent contains persistent compounds or the toxicity persists), AF = 0.01; if non-persistant, AF = 0.05. Thus,

pass

non-persistent $IWC \geq LC50 \times 0.05$
persistent $IWC \geq LC50 \times 0.01$

fail

non-persistent $IWC < LC50 \times 0.05$
persistent $IWC < LC50 \times 0.01$

Example

For example, a bioassay of an electroplating may show that a mixture of 10% effluent and 90% dilution water kills half of the test organisms. The LC50 is 10. Since the primary cause of toxicity is probably metals, the toxicity of the waste should be considered persistent; thus the application factor is .01. The product of these is 0.10 x .01 = 0.001. If the dilution of the effluent is greater than one thousand fold, the effluent should pass the evaluation. If the dilution is less

than 1000, additional treatment may be needed. For lakes or estuaries, a mixing zone volume is used instead of the 7 day, 10 year low flow.

Reporting Results

C. Inspection Report

Handout 15 & 17: Instruction for Completing Forms

The Acute Toxicity Laboratory Evaluation form and the NPDES Compliance Inspection Report should be filled out according to the instructions provided in Handout 15 and 17. Each trainee should review the instructions prior to filling them out.

Although biomonitoring is not currently required under the NPDES Program, it undoubtedly will be in the future. Thus, it is important for NPDES inspectors to be thoroughly familiar with the procedures for conducting a biomonitoring inspection and performing subsequent toxicity tests.

HANDOUT 1

GLOSSARY

Acclimation -- The process of adjusting to a new environment. The test organisms should be acclimated to the dilution water before the test begins.

Acute Toxicity -- Short-term effect of a toxicant on test organisms. Death is the end point in acute toxicity tests.

Acute Toxicity Testing -- Short term bioassays to evaluate lethal effects.

Announced Inspection -- An inspection in which the permittee is informed of the exact dates the inspection will be conducted.

Audit (or Performance Audit Inspection - PAI) -- A nonsampling type of inspection to assess all the elements of a permittee's self-monitoring program, such as testing procedures and methodology while being implemented, quality assurance, data gathering and interpretation, files, and laboratory data facilities.

Bioassay -- A test to detect or measure the effect of one or more substance, waste, or environmental factors on living organisms.

Effluent Biomonitoring -- Measurements of the biological effect of effluents (such as toxicity, biostimulation, and bioaccummulation) and their effect on the abundance, composition, and functions of indigenous aquatic organisms in receiving waters (biological integrity). For purposes of this manual, effluent biomonitoring refers to acute toxicity bioassays.

Definitive test -- A full-scale bioassay consisting of at least five different concentrations of effluent and a control each containing 20 or more organisms of a given species.

Dilution Water -- Mixing water to be used for preparing dilutions of the effluent. This water is usually collected from a point as close as possible but outside the zone of influence of the effluent. This water is also used for the control test chambers.

EC50 -- Median effective concentration; the concentration producing a specific response, other than death, in 50% of the test organisms. Responses can be behavioral, a developmental abnormality, or a deformity.

Effluent -- For the purposes of this module, an outflow from a point source which is regulated by an NPDES permit.

Evaluation Inspections -- Inspections that do not include any type of sampling of the effluent or the receiving waters. These inspections assess some aspects of a permittee's self-monitoring program.

Flowthrough Bioassay -- Continous flow bioassay; the type of test where different concentrations of the effluent are prepared by mixing with adequate quality dilution water and are then tested by allowing the effluent mixtures to flow at predetermined rates into chambers containing the text organism.

LC50 -- Median lethal concentration. The concentration which kills 50% of the test organisms.

Rangefinding Test -- A short-term (8-24 hours) flowthrough or static bioassy (usually static) used for determining the approximate concentrations, above and below the LC50, to be used in the definitive test. In this test, groups of five organisms are exposed to from three to five widely spaced effluent dilutions.

Sampling Point -- Particular site whose location may be specified in a permit and from which effluent samples are to be collected for testing and evaluation.

Standard Toxicant -- A reference toxicant used for quality assurance purposes in the biomonitoring program. It is used in a bioassay to determine the reproducibility of test results and differences in senstivity among batches of test organisms.

Point Source Categories

1. TIMBER PRODUCTS PROCESSING

SIC 2411 - Logging Camps and Logging Contractors (Camps Only)
SIC 2421 - Saw Mills and Planing Mills, General
SIC 2426 - Hardwood Dimension and Flooring Mills
SIC 2429 - Special Purpose Sawmills, Not Elsewhere Classified
SIC 2431 - Millwork
SIC 2434 - Wood Kitchen Cabinets
SIC 2435 - Hardwood Veneer and Plywood
SIC 2436 - Softwood Veneer and Plywood
SIC 2439 - Structural Wood Members, Not Elsewhere Classified
SIC 2491 - Wood Preserving
SIC 2499 - Wood Products, Not Elsewhere Classified (Furniture Mills)
SIC 2661 - Building Paper and Building Board Mills (Hardboard Only)

2. STEAM ELECTRIC POWER PLANTS

SIC 4911 - Electric Services (Limited to Steam-Electric Power Plants)

3. LEATHER TANNING AND FINISHING

SIC 31 - Leather and Leather Products

4. IRON AND STEEL MANUFACTURING

SIC 3312 - Blast Furnaces (Including Coke Ovens), Steel Works and Rolling Mills.
SIC 3313 - Electrometallurgical Products.
SIC 3315 - Steel Wire Drawing and Steel Nails and Spikes.
SIC 3316 - Cold Rolled Steel Sheet, Strip and Bars.
SIC 3317 - Steel Pipe and Tubes.

5. PETROLEUM REFINING

SIC 2911 - Petroleum Refining (Including 1) Topping Plant; 2) Topping and Cracking Plants; 3) Topping, Cracking and Petro-chemical Plants; 4) Integrated Plants; and, 5) Integrated and Petro-chemical Plants)

6. INORGANIC CHEMICALS MANUFACTURING

SIC 2812 - Alkalies and Chlorine
SIC 2813 - Industrial Gasses
SIC 2816 - Inorganic Pigments
SIC 2819 - Industrial Inorganic Chemicals, Not Elsewhere Classified

7. TEXTILE MILLS

SIC 22 - Textile Mill Products
SIC 23 - Apparel and Other Finished Products Made from Fabrics and Similar Materials

8. ORGANIC CHEMICALS MANUFACTURING

SIC 2865 - Cylic (Coal Tar) Crudes, and Cylic Intermediates, Dyes, and Organic Pigments (Lakes and Toners)
SIC 2869 - Industrial Organic Chemicals, Not Elsewhere Classified

9. NONFERROUS METALS MANUFACTURING

SIC 2819 - Industrial Inorganic Chemicals, Not Elsewhere Classified (Bauxite Refining Only)
SIC 3331 - Primary Smelting and Refining of Copper
SIC 3332 - Primary Smelting and Refining of Lead
SIC 3333 - Primary Smelting and Refining of Zinc
SIC 3334 - Primary Production of Aluminum
SIC 3339 - Primary Smelting and Refining of Nonferrous Metals, Not Elsewhere Classified
SIC 3341 - Secondary Smelting and Refining of Nonferrous Metals

10. PAVING AND ROOFING MATERIALS (TARS AND ASPHALT)

SIC 2951 - Paving Mixtures and Blocks
SIC 2952 - Asphalt Felts and Coatings
SIC 3996 - Linoleum, Asphalted-Felt-Base, and Other Hard Surface Floor Coverings, Not Elsewhere Classified

11. PAINT AND INK FORMULATION AND PRINTING

SIC 2711 - Newspapers: Publishing, Publishing and Printing
SIC 2721 - Periodicals: Publishing, Publishing and Printing
SIC 2731 - Books: Publishing, Publishing and Printing
SIC 2732 - Book Printing
SIC 2741 - Miscellaneous Publishing
SIC 2751 - Commercial Printing, Letterpress and Screen
SIC 2752 - Commercial Printing, Letterpress and Lithographic
SIC 2753 - Engraving and Plate Printing
SIC 2754 - Commercial Printing, Gravure
SIC 2761 - Manifold Business Forms
SIC 2771 - Greeting Card Publishing
SIC 2793 - Photoengraving
SIC 2794 - Electrotyping and Stereotyping

SIC 2795 - Lithographic Platemaking and Related Services
SIC 2851 - Paints, Varnishes, Lacquers, Enamels, and Allied Products
SIC 2893 - Printing Ink
SIC 3951 - Pens, Mechanical pencils, and Parts and Stamp Pads (Inked Materials Only)
SIC 3952 - Lead Pencils, Crayons, and Artists' Materials
SIC 3955 - Carbon Paper and Inked Ribbons

12. SOAP AND DETERGENT MANUFACTURING

SIC 2841 - Soap and Other Detergents, except Specialty Cleaners

13. AUTO AND OTHER LAUNDRIES

SIC 7211 - Power Laundries, Family and Commercial
SIC 7213 - Linen Supply
SIC 7214 - Diaper Service
SIC 7215 - Coin-operated Laundries and Dry Cleaning
SIC 7216 - Dry Cleaning Plants, Except Rug Cleaning
SIC 7217 - Carpet and Upholstery Cleaning
SIC 7218 - Industrial Laundries
SIC 7219 - Laundry and Garment Services, Not Elsewhere Classified
None - Auto Wash Establishments

14. PLASTIC AND SYNTHETIC MATERIALS MANUFACTURING

SIC 282 - Plastic Materials and Synthetic Resins, Synthetic and Other Manmade Fibers, except Glass

15. PULP AND PAPERBOARD MILLS; AND CONVERTED PAPER PRODUCTS

SIC 2611 - Pulp Mills
SIC 2621 - Paper Mills, except Building Paper Mills
SIC 2631 - Paperboard Mills
SIC 2641 - Paper Coating and Glazing
SIC 2642 - Envelopes
SIC 2643 - Bags, Except Textile Bags
SIC 2645 - Die-Cut Paper and Paperboard and Cardboard
SIC 2646 - Pressed and Molded Pulp Goods
SIC 2647 - Sanitary Paper Products
SIC 2648 - Stationery, Tablets, and Related Products
SIC 2649 - Converted Paper and Paperboard Products, Not Elsewhere Classified
SIC 2651 - Folding Paperboard Boxes
SIC 2652 - Set-up Paperboard Boxes
SIC 2653 - Corrugated and Solid Fiber Boxes
SIC 2654 - Sanitary Food Containers
SIC 2655 - Fiber Cans, Tubes, Drums, and Similar Products
SIC 2661 - Building Paper and Building Board Mills
SIC 2782 - Blankbooks, Looseleaf Binders and Devices

16. RUBBER PROCESSING

SIC 2822 - Synthetic Rubber (Vulcanizable Elastomers)
SIC 2891 - Rubber Cement

SIC 3011 - Tires and Inner Tubes
SIC 3021 - Rubber and Plastics Footwear (Rubber Only)
SIC 3031 - Reclaimed Rubber
SIC 3041 - Rubber and Plastics Hose and Belting (Rubber Only)
SIC 3069 - Fabricated Rubber Products, Not Elsewhere Classified
SIC 3293 - Gaskets, Packing, and Sealing Devices (Rubber Packing Only)

17. MISCELLANEOUS CHEMICALS

SIC 2831 - Biological Products
SIC 2833 - Medicinal Chemicals and Botanical Products
SIC 2834 - Pharmaceutical Preparations
SIC 2861 - Gum and Wood Chemicals
SIC 2879 - Pesticides and Agricultural Chemicals, Not Elsewhere Classified
SIC 2891 - Adhesive and Sealants
SIC 2892 - Explosives
SIC 2895 - Carbon Black
SIC 2899 - Chemicals and Chemical Preparation, Not Elsewhere Classified
SIC 3861 - Photographic Equipment and Supplies

18. MACHINERY AND MECHANICAL PRODUCTS MANUFACTURING

SIC 3021 - Rubber and Plastics Footwear (Balance)
SIC 3041 - Rubber and Plastics Hose and Belting (Balance)
SIC 3079 - Miscellaneous Plastics Products
SIC 3293 - Gaskets, Packing, and Sealing Devices (Balance)
SIC 3321 - Gray Iron Foundries
SIC 3322 - Malleable Iron Foundries
SIC 3324 - Steel Investment Foundries
SIC 3325 - Steel Foundries, Not Elsewhere Classified
SIC 3351 - Rolling, Drawing, and Extruding of Copper
SIC 3353 - Aluminum Sheet, Plate, and Foil
SIC 3354 - Aluminum Extruded Products
SIC 3355 - Aluminum Rolling and Drawing, Not Elsewhere Classified
SIC 3356 - Rolling, Drawing, and Extruding of Nonferrous Metals, except copper and aluminum
SIC 3357 - Drawing and Insulating of Nonferrous Wire
SIC 3361 - Aluminum Foundries (Castings)
SIC 3362 - Brass, Bronze, Copper, Copper Base Alloy Foundries (Castings)
SIC 3369 - Nonferrous Foundries (Castings), Not Elsewhere Classified
SIC 3398 - Metal Heat Treating
SIC 3399 - Primary Metal Products, Not Elsewhere Classified
SIC 3411 - Metal Cans
SIC 3412 - Metal Shipping Barrels, Drums, Kegs, and Pails
SIC 3421 - Cutlery
SIC 3423 - Hand and Edge Tools, Except Machine Tools and Hand Saws
SIC 3425 - Hand Saws and Saw Blades
SIC 3429 - Hardware, Not Elsewhere Classified
SIC 3431 - Enameled Iron and Metal Sanitary Ware
SIC 3432 - Plumbing Fixture Fittings and Trim (Brass Goods)
SIC 3433 - Heating Equipment, Except Electric and Warm Air Furnaces
SIC 3441 - Fabricated Structural Metal
SIC 3442 - Metal Doors, Sash, Frames, Molding, and Trim

SIC 3443 - Fabricated Platework (Boiler Shops)
SIC 3444 - Sheet Metal Work
SIC 3446 - Architectural and Ornamental Metal Work
SIC 3448 - Prefabricated Metal Buildings and Components
SIC 3449 - Miscellaneous Metal Work
SIC 3451 - Screw Machine Products
SIC 3452 - Bolts, Nuts, Screws, Rivets, and Washers
SIC 3462 - Iron and Steel Forgings
SIC 3463 - Nonferrous Forgings
SIC 3465 - Automotive Stampings
SIC 3466 - Crowns and Closures
SIC 3469 - Metal Stampings, Not Elsewhere Classified
SIC 3482 - Small Arms Ammunition
SIC 3483 - Ammunition, Except for Small Arms, Not Elsewhere Classified
SIC 3484 - Small Arms
SIC 3489 - Ordnance and Accessories, Not Elsewhere Classified
SIC 3493 - Steel Springs, Except Wire
SIC 3494 - Valves and Pipe Fittings, Except Plumbers' Brass Goods
SIC 3495 - Wire Springs
SIC 3496 - Miscellaneous Fabricated Wire Products
SIC 3497 - Metal Foil and Leaf
SIC 3498 - Fabricated Pipe and Fabricated Pipe Fittings
SIC 3499 - Fabricated Metal Products, Not Elsewhere Classified
SIC 3511 - Steam, Gas, and Hydraulic Turbines and Turbine Generator Set Units
SIC 3519 - Internal Combustion Engines, Not Elsewhere Classified
SIC 3523 - Farm Machinery and Equipment
SIC 3524 - Garden Tractors and Lawn and Garden Equipment
SIC 3531 - Construction Machinery and Equipment
SIC 3532 - Mining Machinery and Equipment, Except Oil Field Machinery and Equipment
SIC 3533 - Oil Field Machinery and Equipment
SIC 3534 - Elevators and Moving Stairways
SIC 3535 - Conveyors and Conveying Equipment
SIC 3536 - Hoists, Industrial Cranes, and Monorail Systems
SIC 3537 - Industrial Trucks, Tractors, Trailers, and Stackers
SIC 3541 - Machine Tools, Metal Cutting Types
SIC 3542 - Machine Tools, Metal Forming Types
SIC 3544 - Special Dies and Tools, Die Sets, Jigs and Fixtures and Industrial Molds
SIC 3545 - Machine Tool Accessories and Measuring Devices
SIC 3546 - Power Driven Hand Tools
SIC 3547 - Rolling Mill Machinery and Equipment
SIC 3549 - Metalworking Machinery, Not Elsewhere Classified
SIC 3551 - Food Products Machinery
SIC 3552 - Textile Machinery
SIC 3553 - Woodworking Machinery
SIC 3554 - Paper Industries Machinery
SIC 3555 - Printing Trades Machinery and Equipment
SIC 3559 - Special Industry Machinery, Not Elsewhere Classified
SIC 3561 - Pumps and Pumping Equipment
SIC 3562 - Ball and Roller Bearings
SIC 3563 - Air and Gas Compressors
SIC 3564 - Blowers and Exhaust and Ventilation Fans
SIC 3565 - Industrial Patterns

SIC 3566 - Speed Changers, Industrial High Speed Drives, and Gears
SIC 3567 - Industrial Process Furnaces and Ovens
SIC 3568 - Mechanical Power Transmission Equipment, Not Elsewhere Classifie
SIC 3569 - General Industrial Machinery and Equipment, Not Elsewhere Classified
SIC 3572 - Typewriters
SIC 3573 - Electronic Computing Equipment
SIC 3574 - Calculating and Accounting Machines, Except Electronic Computing Equipment
SIC 3576 - Scales and Balances, Except Laboratory
SIC 3579 - Office Machines, Not Elsewhere Classified
SIC 3581 - Automatic Merchandising Machines
SIC 3582 - Commercial Laundry, Dry Cleaning, and Pressing Machines
SIC 3585 - Air Conditioning and Warm Air Heating Equipment and Commercial and Industrial Refrigeration Equipment
SIC 3586 - Measuring and Dispensing Pumps
SIC 3589 - Service Industry Machines, Not Elsewhere Classified
SIC 3592 - Carburetors, Piston, Piston Rings, and Valves
SIC 3599 - Machinery, Except Electrical, Not Elsewhere Classified
SIC 3612 - Power, Distribution, and Specialty Transformers
SIC 3613 - Switchgear and Switchboard Apparatus
SIC 3621 - Motors and Generators
SIC 3622 - Industrial Controls
SIC 3623 - Welding Apparatus, Electric
SIC 3624 - Carbon and Graphite Products
SIC 3629 - Electrical Industrial Apparatus, Not Elsewhere Classified
SIC 3631 - Household Cooking Equipment
SIC 3632 - Household Refrigerators and Home and Farm Freezers
SIC 3633 - Household Laundry Equipment
SIC 3634 - Electric Housewares and Fans
SIC 3635 - Household Vacuum Cleaners
SIC 3639 - Household Appliances, Not Elsewhere Classified
SIC 3641 - Electric Lamps
SIC 3643 - Current-Carrying Wiring Devices
SIC 3644 - Noncurrent-Carrying Wiring Devices
SIC 3645 - Residential Electric Lighting Fixtures
SIC 3646 - Commercial, Industrial, and Institutional Electric Lighting Fixtures
SIC 3647 - Vehicular Lighting Equipment
SIC 3648 - Lighting Equipment, Not Elsewhere Classified
SIC 3651 - Radio and Television Receiving Sets, Except Communication Types
SIC 3652 - Phonograph Records and Pre-recorded Magnetic Tape
SIC 3661 - Telephone and Telegraph Apparatus
SIC 3662 - Radio and Television Transmitting, Signaling, and Detection Equipment and Apparatus
SIC 3671 - Radio and Television Receiving Type Electron Tubes, Except Cathode Ray
SIC 3672 - Cathode Ray Television Picture Tubes
SIC 3673 - Transmitting, Industrial, and Special Purpose Electron Tubes
SIC 3674 - Semiconductors and Related Devices
SIC 3675 - Electronic Capacitors
SIC 3676 - Resistors, for Electronic Applications
SIC 3677 - Electronic Coils, Transformers and Other Inductors
SIC 3678 - Connectors, for Electronic Applications

SIC 3679 - Electronic Components, Not Elsewhere Classified
SIC 3691 - Storage Batteries
SIC 3692 - Primary Batteries, Dry and Wet
SIC 3693 - Radiographic X-ray, Fluoroscopic X-ray, Therapeutic X-ray, and Other X-ray Apparatus and Tubes; Electromedical and Electrotherapeutic Apparatus
SIC 3694 - Electrical Equipment for Internal Combustion Engines
SIC 3699 - Electrical Machinery, Equipment, and Supplies, Not Elsewhere Classified
SIC 3711 - Motor Vehicles and Passenger Car Bodies
SIC 3713 - Truck and Bus Bodies
SIC 3714 - Motor Vehicle Parts and Accessories
SIC 3715 - Truck Trailers
SIC 3721 - Aircraft
SIC 3724 - Aircraft Engines and Engine Parts
SIC 3728 - Aircraft Parts and Auxiliary Equipment, Not Elsewhere Classified
SIC 3731 - Ship Building and Repairing
SIC 3732 - Boat Building and Repairing
SIC 3743 - Railroad Equipment
SIC 3751 - Motorcycles, Bicycles, and Parts
SIC 3761 - Guided Missiles and Space Vehicles
SIC 3764 - Guided Missile and Space Vehicle Propulsion Units and Propulsion Unit Parts
SIC 3769 - Guided Missile and Space Vehicle Parts and Auxiliary Equipment, Not Elsewhere Classified
SIC 3792 - Travel Trailers and Campers
SIC 3795 - Tanks and Tank Components
SIC 3799 - Transportation Equipment, Not Elsewhere Classified
SIC 3811 - Engineering, Laboratory, Scientific, and Research Instruments and Associated Equipment
SIC 3822 - Automatic Controls for Regulating Residential and Commercial Environments and Appliances
SIC 3823 - Industrial Instruments for Measurement, Display and Control of Process Variables; and Related Products
SIC 3824 - Totalizing Fluid Meters and Counting Devices
SIC 3825 - Instruments for Measuring and Testing of Electricity and Electrical Signals
SIC 3829 - Measuring and Controlling Devices, Not Elsewhere Classified
SIC 3832 - Optical Instruments and Lenses
SIC 3841 - Surgical and Medical Instruments and Apparatus
SIC 3842 - Orthopedic, Prosthetic, and Surgical Appliances and Supplies
SIC 3843 - Dental Equipment and Supplies
SIC 3851 - Ophthalmic Goods
SIC 3873 - Watches, Clocks, Clockwork Operated Devices and Parts
SIC 3911 - Jewelry, Precious Metal
SIC 3914 - Silverware, Plated Ware, and Stainless Steel Ware
SIC 3915 - Jewelers' Findings and Materials, and Lapidary Work
SIC 3931 - Musical Instruments
SIC 3942 - Dolls
SIC 3944 - Games, Toys, and Children's Vehicles; Except Dolls and Bicycles
SIC 3949 - Sporting and Athletic Goods, Not Elsewhere Classified
SIC 3951 - Pens, Mechanical Pencils, and Parts (Balance)
SIC 3961 - Costume Jewelry and Costume Novelties, Except Precious Metal

SIC 3991 - Brooms and Brushes
SIC 3993 - Signs and Advertising Displays
SIC 3995 - Burial Caskets

19. ELECTROPLATING

SIC 347 - Coating, Engraving, and Allied Services

20. ORE MINING AND DRESSING

SIC 1011 - Iron Ores
SIC 1021 - Copper Ores
SIC 1031 - Lead and Zinc Ores
SIC 1041 - Gold Ores
SIC 1044 - Silver Ores
SIC 1051 - Bauxite and Other Aluminum Ores
SIC 1061 - Ferroalloy Ores, Except Vanadium
SIC 1092 - Mercury Ores
SIC 1094 - Uranium-Radium-Vanadium Ores
SIC 1099 - Metal Ores, Not Elsewhere Classified

21. COAL MINING

SIC 1111 - Anthracite
SIC 1112 - Anthracite Mining Services
SIC 1211 - Bituminous Coal and Lignite
SIC 1213 - Bituminous Coal and Lignite Mining Services

THE 129 PRIORITY TOXIC POLLUTANTS

Pollutant	Characteristics	Sources	Remarks
Pesticides Generally chlorinated hydrocarbons	Readily assimilated by aquatic animals, fat soluble, concentrated through the food chain (biomagnified), persistent in soil and sediments	Direct application to farm- and forestlands, runoff from lawns and gardens, urban runoff, discharge in industrial wastewater	Several chlorinated hydrocarbon pesticides already restricted by EPA; aldrin, dieldrin, DDT, DDD, endrin, heptachlor, lindane, and chlordane
Polychlorinated biphenyls (PCBs) Used in electrical capacitors and transformers, paints, plastics, insecticides, other industrial products	Readily assimilated by aquatic animals, fat soluble, subject to biomagnification, persistent, chemically similar to the chlorinated hydrocarbons	Municipal and industrial waste discharges disposed of in dumps and landfills	TSCA ban on production after 6/1/79 but will persist in sediments; restrictions on many freshwater fisheries as a result of PCB pollution (e.g., lower Hudson, upper Housatonic, parts of Lake Michigan)
Metals Antimony, arsenic, beryllium, cadmium, copper, lead, mercury, nickel, selenium, silver, thallium, and zinc *Other inorganics* Asbestos and cyanide	Not biodegradable, persistent in sediments, toxic in solution, subject to biomagnification *Asbestos* May cause cancer when inhaled, aquatic toxicity not well understood *Cyanide* Variably persistent, inhibits oxygen metabolism	Industrial discharges, mining activity, urban runoff, erosion of metal-rich soil, certain agricultural uses (e.g., mercury as a fungicide) *Asbestos* Manufacture and use as a retardant, roofing material, brake lining, etc.; runoff from mining *Cyanide* Wide variety of industrial uses	
Halogenated aliphatics Used in fire extinguishers, refrigerants, propellants, pesticides, solvents for oils, and greases and in dry cleaning	Largest single class of "priority toxics," can cause damage to central nervous system and liver, not very persistent	Produced by chlorination of water, vaporization during use	Large volume industrial chemicals, widely dispersed, but less threat to the environment than persistent chemicals
Ethers Used mainly as solvents for polymer plastics	Potent carcinogen, aquatic toxicity and fate not well understood	Escape during production and use	Though some are volatile, ethers have been identified in some natural waters.
Phthalate esters Used chiefly in production of polyvinyl chloride and thermoplastics as plasticizers	Common aquatic pollutant, moderately toxic but teratogenic and mutagenic properties in low concentrations; aquatic invertebrates are particularly sensitive to toxic effects; persistent; and can be biomagnified	Waste disposal vaporization during use (in nonplastics)	
Monocyclic aromatics (excluding phenols, cresols and phthalates) Used in the manufacture of other chemicals, explosives, dyes and pigments, and in solvents, fungicides, and herbicides	Central nervous system depressant; can damage liver and kidneys	Enter environment during production and byproduct production states by direct volatization, wastewater	
Phenols Large volume industrial compounds used chiefly as chemical intermediates in the production of synthetic polymers, dyestuffs, pigments, pesticides, and herbicides	Toxicity increases with degree of chlorination of the phenolic molecule; very low concentrations can taint fish flesh and impart objectionable odor and taste to drinking water; difficult to remove from water by conventional treatment; carcinogenic in mice	Occur naturally in fossil fuels, waste water from coking ovens, oil refineries, tar distillation plants, herbicide manufacturing, and plastic manufacturing; can all contain phenolic compounds	
Polycyclic aromatic hydrocarbons Used as dyestuffs, chemical intermediates, pesticides, herbicides, motor fuels, and oils	Carcinogenic in animals and indirectly linked to cancer in humans; most work done on air pollution; more is needed on the aquatic toxicity of these compounds; not persistent and are biodegradable though bioaccumulation can occur	Fossil fuels (use, spills, and production), incomplete combustion of hydrocarbons	
Nitrosamines Used in the production of organic chemicals and rubber; patents exist on processes using these compounds.	Tests on laboratory animals have shown the nitrosamines to be some of the most potent carcinogens.	Production and use can occur spontaneously in food cooking operations	

Reference: Council of Environmental Quality, Environmental Quality 1978, USGPO #041-11-0040-8, December, 1978

Recommended List of Priority
Pollutants

Compound Name

1. *acenaphthene
2. *acrolein
3. *acrylonitrile
4. *benzene
5. *benzidene
6. *carbon tetrachloride (tetrachloromethane)

*Chlorinated benzenes (other than dichlorobenzenes)

7. chlorobenezene
8. 1,2,4-trichlorobenzene
9. hexachlorobenzene

*Chlorinated ethanes (including 1,2-dichloroethane, 1,1,1-trichloroethane and hexachloroethane)

10. 1,2-dichloroethane
11. 1,1,1-trichloroethane
12. hexachloroethane
13. 1,1-dichloroethane
14. 1,1,2-trichloroethane
15. 1,1,2,2-tetrachloroethane
16. chloroethane

*Chloroalkyl ethers (chloromethyl, chloroethyl and mixed ethers)

17. bis (chloromethyl) ether
18. bis (2-chloroethly) ether
19. 2-chloroethyl vinyl ether (mixed)

*Chlorinated naphtalene

20. 2-chloronaphthalene

*Chlorinated phenols (other than those listed elsewhere; includes trichlorophenols and chlorinated cresols)

21. 2,4,6- trichlorophenol
22. parachlorometa cresol
23. *chloroform (trichloromethane)
24. *2-chlorophenol

*Dichlorobenzenes

25. 1,2-dichlorobenzene
26. 1,3-dichlorobenzene
27. 1,4-dichlorobenzene

*Dichlorobenzidine

28. 3,3'-dichlorobenzidine

*Dichloroethylenes (1,1-dichloroethylene and 1,2-dichloroethylene)

29. 1,1-dichloroethylene
30. 1,2-trans-dichloroethylene
31. *2,4-dichlorophenol

*Dichloropropane and dichloropropene

32. 1,2-dichloropropane
33. 1,2-dichloropropylene (1,3-dichloropropene)
34. *2,4-dimethylphenol

*Dinitrotoluene

35. 2,4-dinitrotoluene
36. 2,6,-dinitrotoluene
37. *1,2-diphenylhydrazine
38. *ethylbenzene
39 *fluoranthene

*Haloethers (other than those listed elsewhere)

40. 4-chlorophenyl phenyl ether
41. 4-bromophenyl phenyl ether
42. bis(2-chloroisopropyl) ether
43. bis(2-chloroethoxy) methane

*Specific compounds and chemical classes as listed in the consent degree.

*Halomethanes (other than those listed elsewhere

44. methylene chloride (dichloromethane)
45. methyl chloride (chloromethane)
46. methyl bromide (bromomethane)
47. bromoform (tribromomethane)
48. dichlorobromomethane
49. trichlorofluoromethane
50. dichlorodifluoromethane
51. chlorodibromomethane
52. *hexachlorobutadiene
53. *hexachlorocyclopentadiene
54. *isophorone
55. *naphthalene
56. *nitrobenzene

*Nitrophenols (including 2,4-dinitrophenol and dinitrocresol)

57. 2-nitrophenol
58. 4-nitrophenol
59. *2,4-dinitrophenol
60. 4,6-dinitro-o-cresol

*Nitrosamines

61. N-nitrosodimethylamine
62 N-nitrosodiphenylamine
63. N-nitrosodi-n-propylamine
64. *pentachlorophenol
65. *phenol

*Phthalate esters

66. bis(2-ethylhexyl) phthalate
67. butyl benzyl phthalate
68. di-n-butyl phtalate
69. di-n-octyl phtalate
70. diethyl phtalate
71. dimethyl phthalate

*Polynuclear aromatic hydracarbons

72. benzo (a)anthracene (1,2-benzanthracene)
73. benzo (a) pyrene (3,4-benzopyrene)
74. 3,4-benzofluoranthene .
75. benzo(k)fluoranthane (11,12-benzofluoranthene)
76. chrysene
77. acenaphthylene
78. anthracene
79. benzo(ghi)perylene (1,12-benzoperylene)
80. fluorene
81. phenanthrene
82. dibenzo (a,h)anthracene (1,2,5,6-dibenzanthracene)
83. indeno (1,2,3-cd) pyrene (2,3,-o-phenylenepyrene)
84. pyrene
85. *tetrachloroethylene
86. *toluene
87. *trichloroethylene
88. *vinyl chloride (chloroethylene)

Pesticides and Metabolites

89. *aldrin
90. *dieldrin
91. *chlordane (technical mixture & metabolites)

*DDT and metabolites

92. 4,4'-DDT
93. 4,4'-DDE(p,p'DDX)
94. 4,4'-DDD(p,p'TDE)

*endosulfan and metabolites

95. a-endosulfan-Alpha
96. b-endosulfan-Beta
97. endosulfan sulfate

*endrin and metabolites

98. endrin
99. endrin aldehyde

*heptachlor and metabolites

100. heptachlor
101. heptachlor epoxide

*hexachlorocyclohexane (all isomers)

102. a-BHC-Alpha
103. b-BHC-Beta
104. r-BHC (lindane)-Gamma
105. g-BHC-Delta

*polychlorinated biphenyls (PCB's)

106. PCB-1242 (Arochlor 1242)
107. PCB-1254 (Arochlor 1254)
108. PCB-1221 (Arochlor 1221)
109. PCB-1232 (Arochlor 1232)
110. PCB-1248 (Arochlor 1248)
111. PCB-1260 (Arochlor 1260)
112. PCB-1016 (Arochlor 1016)
113. *Toxaphene
114. *Antimony (Total)
115. *Arsenic (Total)

116. *Asbestos (Fibrous)
117. *Beryllium (Total)
118. *Cadmium (Total)
119. *Chromium (Total)
120. *Copper (Total)
121. *Cyanide (Total)
122. *Lead (Total)
123. *Mercury (Total)
124. *Nickel (Total)
125. *Selenium (Total)
126. *Silver (Total)
127. *Thallium (Total)
128. *Zinc (Total)
129. **2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

** This compound was specifically listed in the consent degree. Because of the extreme toxicity (TCDD). We are recommending that laboratories not acquire analytical standard for the compound.

SECTION 308(a) OF THE CLEAN WATER ACT

Section 308(a) of the Act states in part:

(A) the Administrator shall require the owner or operator of any point source to (i) establish and maintain such records, (ii) make such reports, (iii) install, use and maintain such monitoring equipment or methods (including where appropriate, biological monitoring methods), (iv) sample such effluents (in accordance with such methods, at such locations, at such intervals, and in such manner as the Administrator shall prescribe), and (v) provide such other information as he may reasonably require; and

(B) the Administrator or his authorized representative upon presentation of his credentials--
(i) shall have a right of entry to, upon or through any premises in which an effluent source is located or in which any records required to be maintained under clause (A) of this subsection are located, and
(ii) may at reasonable times have access to and copy any records, inspect any monitoring equipment or method required under clause (A), and sample any effluents which the owner or operator of such source is required to sample under such clause.

TABLE 1. RECOMMENDED SPECIES AND TEST TEMPERATURES

Species	Test Temperature (°C)[a]
Freshwater	
Vertebrates	
Coho salmon, *Oncorhynchus kisutch*	12
Rainbow trout, *Salmo gairdneri*	12
Brook trout, *Salvelinus fontinalis*	12
Goldfish, *Carassius auratus*	22
Fathead minnow, *Pimephales promelas*	22
Channel catfish, *Ictalurus punctatus*	22
Bluegill, *Lepomis macrochirus*	22
Invertebrates[a]	
Daphnids, *Daphnia magna* or *D. pulex*	17
Amphipods, *Gammarus lacustris*, *G. fasciatus*, or *G. pseudolimnaeus*	17
Crayfish, *Orconectes* sp., *Cambarus* sp., *Procambarus* sp., or *Pacifastacus leniusculus*	17
Stoneflies, *Pteronarcys* sp.	22
Mayflies, *Baetis* sp. or *Ephemerella* sp.	22
Hexagenia limbata or *H. bilineata*	12
Midges, *Chironomus* sp.	17
	22
	22
Marine and estuarine	
Vertebrates	
Sheepshead minnow, *Cyprinodon variegatus*	22
Mummichog, *Fundulus heteroclitus*	22
Longnose killifish, *Fundulus similis*	22
Silverside, *Menidia* sp.	22
Threespine stickleback, *Casterosteus aculeatus*	22
Pinfish, *Lagodon rhomboides*	22
Spot, *Leiostomus xanthurus*	22
Shiner perch, *Cymatogaster aggregata*	22
Pacific staghorn sculpin, *Leptocottus armatus*	12
Sanddab, *Citharichthys stigmaeus*	12
Flounder, *Paralichthys dentatus*, *P. lethostigma*	12
English sole, *Parophrys vetulus*	22
	12

TABLE 1. (Cont'd)

Species	Test Temperature (°C)[a]
Marine and estuarine Invertebrates[a]	
Shrimp, *Penaeus setiferus*, *P. duorarum*, or *P. aztecus*	22
Grass shrimp, *Palaemonetes* sp.	22
Shrimp, *Crangon* sp.	22
Oceanic shrimp, *Pandalus jordani*	12
Blue crab, *Callinectes sapidus*	22
Dungeness crab, *Cancer magister*	12
Mysid shrimp, *Mysidopsis* sp., *Neomysis* sp.	22
Atlantic oyster, *Crassostrea virginica*	22
Pacific oyster, *Crassostrea gigas*	20

[a]Freshwater amphipods, daphnids, and midge larvae and shrimp should be cultured and tested at the recommended test temperature. Other invertebrates should be held and tested within 5°C of the temperature of the water from which they were obtained. If the recommended test temperature is not within this range, they should be tested at the temperature from the series 7, 12, 17, 22, and 27°C that is closest to the recommended test temperature and is within the allowed range.

Mobile Bioassay Equipment Checklist

Permittee _____
Permit No. _____

General Equipment

____ company file
____ data file
____ maps
____ field data sheets
____ chemical analysis request
____ pens
____ pencils
____ marker pens
____ paper
____ clipboard
____ Kim-wipes
____ plastic garbage bags
____ electrical tape
____ tape
____ flashlights
____ camera
____ film
____ rope
____ rain gear
____ waders
____ half boats
____ 250 ft. extension cords (3)
____ short extension cords
____ tools
____ solder and soldering gun
____ silicone glue
____ battery charger
____ jumping cables
____ oil and funnel
____ gas cans (1 N.J.) 2. N.Y.

Chemical Sampling Equipment

____ cube containers - 1 qt.
____ cube containers - 1 gal.
____ cube containers - 2 1/2 gal.
____ TOC test tubes
____ volatile organic jars
____ nitric acid (metals)
____ sulfuric acid (nutrients,TOC)
____ Kemmerer sampler
____ buckets

Chemical Analyses Equipment

____ 1000 ml beakers (7)
____ 4 l. graduate (1)
____ 1 l. graduate (2)
____ 500 ml graduate (2)
____ 250 ml graduate (1)
____ 100 ml graduate (2)
____ 50 ml graduate (2)
____ funnels
____ plastic cups (4.5 oz)
____ stirring bars
____ distilled water
____ composite sampler
____ 3/8" vinyl nalgene tubing
____ ice chest

Hardness

____ buffer solution
____ Eriochrome black T
____ EDTA 0.01M

Residual Chlorine

____ phenylarsene oxide (titrant)
____ pH 4 buffer
____ pH 7 buffer
____ potassium iodide
____ electrolyte tablets

Salinity

____ refractometer

Bioassay Equipment

____ static bioassay jars
____ funnels with screening
____ 3/16" silicone tubing
____ 5/8" silicone tubing
____ silent giants
____ oxygen tank
____ air stones
____ 1/8" Latex tubing
____ specimen jars
____ 40% isopropyl
____ submersible pumps (3)
____ dilution water pump
____ black hose line
____ output lines
____ fish food
____ artemia eggs
____ glycerin
____ stoppers

Dissolved Oxygen

____ manganous sulfate
____ alka-azide
____ conc. sulfuric acid
____ sodium thiosulfate
____ starch
____ biiodate
____ 500 ml wide mouth
____ erlenmeyer flask
____ automatic pipetts (3)

Alkalinity and pH

____ 0.02 N sulfuric acid
____ pH meters
____ pH recorder
____ pH 4 buffer
____ pH 7 buffer
____ pH 10 buffer

Handout 8

Preinspection Questions

The following list of questions can be used to establish points of contact, request utilities, and to determine special equipment requirements for on-site flow-through bioassays. Because of the logistics involved, on-site bioassays must be announced by 308 letter. These questions can be posed to the permittee in the 308 letter. The permittee would be requested to answer the following questions:

1. Who is the point of contact in the facility (name and telephone number)?

2. Is electrical power available near the NPDES outfall to operate the trailer (State operating requirements for amperage, voltage, and number of circuits)?

3. Is there space adjacent to the outfall to park a trailer (give dimensions)?

4. What safety equipment are required to enter the facility? Is this supplied? (Indicate the number of people on the inspection team.)

5. What is the source of process water ?

EFFLUENT SAMPLING PROCEDURES

Flowthrough Test

If the industrial or municipal facility discharges continuously, the effluent should be pumped directly and continuously from the discharge line to the dilutor system for the duration of the test. The use of effluent grab samples should be avoided. However, if the effluent cannot be pumped directly and continuously to the dilutor system, the following alternative methods may be employed for collecting the effluent:

(1) When the measured minimum retention time of the effluent is less than 96 hours, a 6-hour composite sample consisting of equal volumes taken every 30 minutes must be collected and transported to the dilutor every 6 hours for the duration of the test.

(2) When the measured minimum retention time of the effluent is between 4 days (96 hours) and 14 days, a 24-hour composite sample consisting of equal volumes taken every hour may be collected daily for the duration of the test.

(3) When the measured minimum retention time of the effluent is greater than 14 days, a single grab sample may be collected daily for the duration of the test.

If the industrial or municipal facility discharges intermittently, i.e., where the waste is discharged over a single 8-hour workshift or is accumulated and discharged at the end of the shift or the week, a composite sample consisting of equal volumes collected every 30 minutes, may be taken for an 8-hour operating shift or for the duration of the plant operating schedule, or a single grab sample may be taken in the case of a batch discharge.

Static Test

If a flowthrough test cannot be used, a static test may be conducted with effluent collected by one of the following methods:

(1) When the measured minimum retention time of the effluent is less than 96 hours, four consecutive 6-hour composite samples, each consisting of equal volumes taken every 30 minutes, are collected and used in setting up four separate static tests.

(2) When the measured minimum retantion time of the effluent is between 4 days (96 hours) and 14 days, a 24-hour composite sample consisting of equal volumes taken every hour is collected daily and used in the test.

(3) When the measured minimum retention time of the effluent is greater than 14 days, a single grab sample may be collected and used in the test.

DEFINITIVE TEST REQUIREMENTS

- Ten organisms of a given species must be present in each test chamber, for a total of twenty per concentration.
- If more than one species are to be used in the test chambers, segregation is necessary.
- There should be randomization of organisms in test chambers.
- Loading must not exceed 5 grams per liter at temperatures of 20°C or less, or 2.5 grams/liter at temperatures above 20°C for flowthrough tests.
- Loading for static tests must not exceed 0.8 grams per liter at temperatures of 20°C or less, and 0.4 grams/liter at temperatures above 20°C.
- Temperatures must be held to within ±2.0°C of the acclimation temperature for both static and flow-through tests.
- Dissolved oxygen concentration should not be permitted to fall below 40 percent saturation for warm water species or 60 percent for cold water species.
- The test begins when the organisms are first exposed to the effluent.
- Organisms are not fed during testing, unless newly hatched or immature.
- The duration of the definitive test is 48 or 96 hours.

DATA SHEET FOR EFFLUENT TOXICITY TEST

Industry/Toxicant _____
Address _____
Contact _____
Effluent Serial Number _____
NPDES Permit Number _____

Beginning: Date _____ Time _____
Ending: Date _____ Time _____
Test Organism _____
Test Temperature Range _____

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)									
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96

HANDOUT 11

LC_{50} DETERMINATION METHODS

A. LITCHFIELD AND WILCOXON ABBREVIATED METHOD OF DETERMINING THE LC50

General Procedure

Step 1: Tabulate the data (see sample data sheet, Fig. 1, p. 33) showing the percent-effluent volumes used, the total number of organisms exposed to each percent-effluent volume, the number of affected organisms, and the observed percent-affected organisms (see Example 1 below). Do not list more than 2 consecutive 100 percent affects at the higher percent-effluent volumes or more than two consecutive 0 percent affects at the lower percent-effluent volumes.

Step 2: Plot the percent-affected organisms against the percent-effluent volume on 2 cycle, logarithmic probability paper (Fig. 2), except for 0 percent or 100 percent affect values. With a straight edge, fit a temporary line through the points, particularly those in the region of 40 percent to 60 percent affects.

Step 3: Using the line drawn through the points, read and list an "expected" percent affect for each percent-effluent volume tested. Disregard the "expected" percent value for any of the percent volumes less than 0.01 or greater than 99.99. Using the expected-percent-affect, calculate from Table 7 a "corrected" value for each 0 percent or 100 percent affect obtained in the test. (Since the expected values in the table are whole numbers, it will be necessary to obtain intermediate values by interpolation.) Plot these values on the logarithmic probability paper (Fig. 2) used in Step 2 and inspect the fit of the line to the completely plotted data. If after plotting the corrected expected values for 0 percent and 100 percent affected, the fit is obviously unsatisfactory, redraw the line and obtain a new set of expected values.

Step 4: List the difference between each observed (or corrected) value and the corresponding expected value. Using each difference and the corresponding expected value, read and list the contributions to Chi-square (Chi^2) from Fig. 3 (a straight edge connecting a value on the Expected-Percent Affected scale with a value on the Observed-Minus-Expected scale, will indicate at the point of intersection of the Chi^2 scale, the contribution to Chi^2. Sum the contributions to Chi^2 and multiply the total by the average number of organisms per effluent volume, i.e., the number of organisms used in K concentrations divided by K, where K is the number of percent-affected organism values plotted. The product is the "calculated" Chi^2 of the line. The degrees of freedom (N) are 2 less than the number of points plotted, i.e., N =K-2. If the calculated Chi^2 is less than the Chi^2 given in Table 8 for N degrees of freedom, the data are non-heterogeneous and the line is a good fit. However, if the calculated Chi^2 is greater than the Chi^2 given in Table 8 for N degrees of freedom, the data are heterogeneous and the line is not a good fit. In the event a line cannot be fitted (the calculated Chi^2 is greater than the tabular Chi^2), the data can not be used to calculate a LC50 or EC50. Litchfield and Wilcoxon provided an alternate method for calculating the 95 percent confidence limits under these circumstances. However, the toxicity test should be repeated.

Step 5: Determine the confidence limits of the LC50.

a. Read from the fitted line (Fig. 2), the percent effluent volumes for the corresponding 16, 50, 84 percent affects (LC16, LC50 and LC84).
b. Calculate the slope function, S, as:

$$S = \frac{LC84/LC50 + LC50/LC16}{2}$$

c. From the tabulation of the data determine N', which is defined as the total number of test organisms used within the percent-affected-organism interval of 16 percent and 84 percent. Calculate the exponent $(2.77/\sqrt{N'})$ for the slope function and the factor, f_{LC50}, used to establish the confidence limits for the LC50 (or EC50).

$$f_{LC50} = S^{(2.77/\sqrt{N'})}$$

The f_{LC50} can be obtained directly from the nomogram in Fig.4 by laying a straight-edge across the appropriate base and exponent values and reading the resultant "f" value.

e. Calculate the confidence limits of the LC50 as follows:

(1) Upper limit for 95% probability = LC50 X f_{LC50}

(2) Lower limit for 95% probability = LC50/f_{LC50}

Example

Steps 1-4: The data were tabulated and plotted (Fig. 2) and the expected values were read from the graph.

STEP ONE				STEP THREE	STEP FOUR	
% Effluent Volume	Number of Organisms	Number of Affected Organisms	Observed % Affected Organisms	Expected % (Fig. 2)	Observed Minus Expected	Chi^2
3.2	20	0	0(.2)[b]	.6	0.4	0.005
5.6	20	1	5	3.5	1.5	0.006
10.0	20	11	55	(14.5)[a]	Aberrant Value	
18.0	20	7	35	38.0	3.0	0.004
32.0	20	12	60	67.0	7.0.	0.024
56.0	20	18	90	87.5	2.5	0.006
100.0	20	20	100 (99.0)[b]	97.0	2.0	0.014
					Total	0.059

a. Percent-affected organisms at the 10 percent effluent volume is obviously an aberrant value and should be omitted when fitting the line in Step 2.

b. Step 3 "Corrected" affected values from Table 7.

Step 4 (Cont.):

Calculation of Chi^2

a. Total number of organisms used in 'K' concentrations = $\frac{120}{6}$ = 20

b. Calculated Chi^2 = 20 x 0.059 = 1.18

c. Degrees of Freedom (N) = K - 2 = 6 - 2 = 4

d. From Table 8, the Chi^2 for 4 degrees of freedom = 9.49

e. The calculated Chi^2 is less than the tabular Chi^2. Therefore, it is assumed the line is a good fit, and the data are non-heterogeneous.

Step 5:

a. From the fitted line in Fig. 2, determine the (percent) effluent concentrations corresponding to the 16%, 50% and 84% affected organism values:

b. LC84 effluent concentrations = 50.0%
LC50 " " = 23.0%
LC16 " " 10.0%

c. Calculate the slope function, 'S', as:

$$S = \frac{LC84/LC50 + LC50/LC16}{2} = \frac{50.0/23.0 + 23.0/10.5}{2}$$

$$= \frac{2.17 + 2.19}{2} = \frac{4.36}{2} = 2.18$$

d. N' = 40 (From Figure 2)

e. Calculate the exponent (N') and factor, f_{LC50}

$$f_{LC50} = S^{2.77/\sqrt{N'}} = 2.18^{2.77/\sqrt{40}} = 2.18^{2.77/6.32} = 2.18^{0.438} = 1.41$$

f. Calculate the confidence limits of the LC50
(1) Upper limit for 95% probability = LC50 X f_{LC50} = 23.0 X 1.4 = 32.2%

(2) Lower limit for 95% probability = LC50/f_{LC50} = 23.0/1.4 = 16.4%

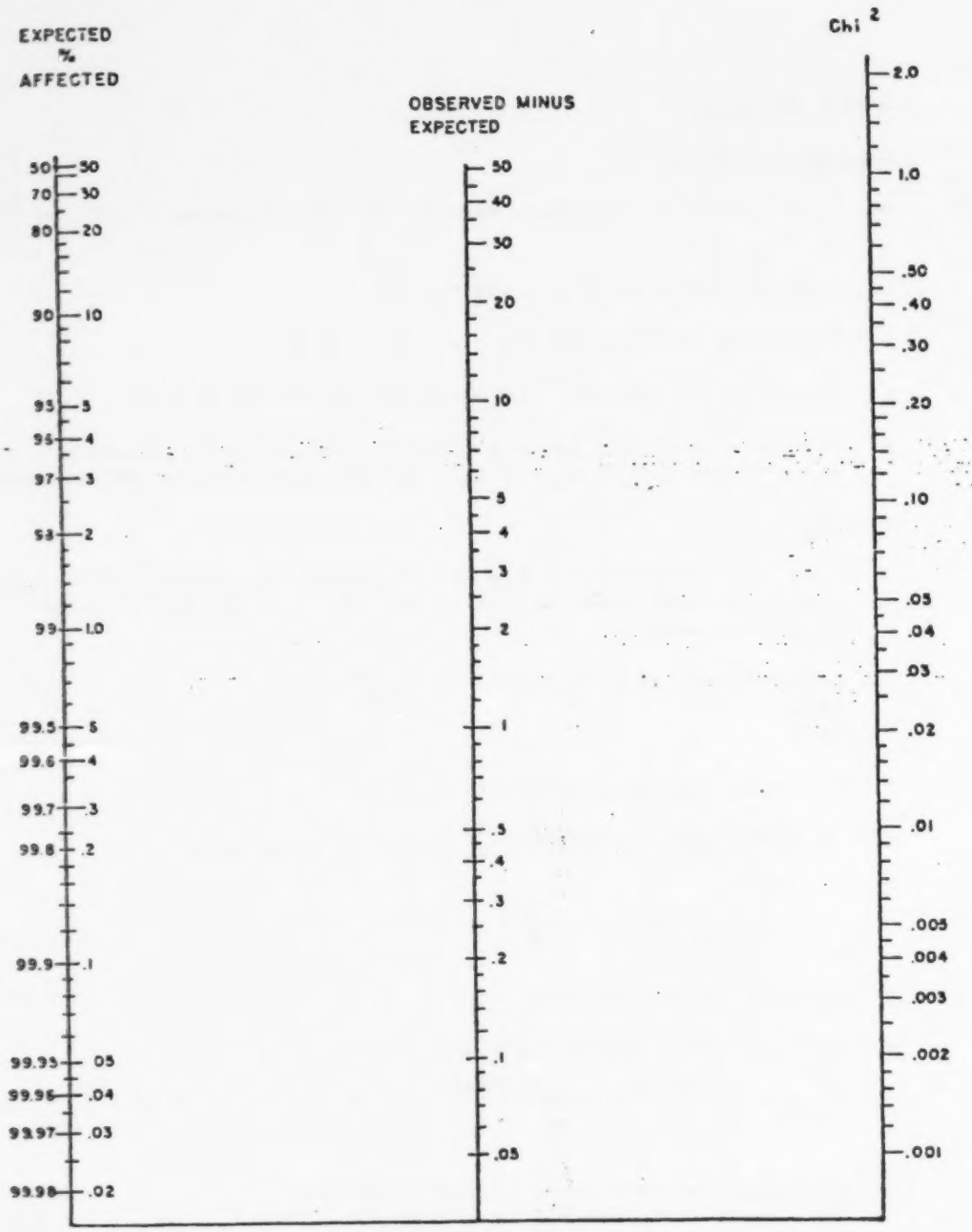

FIG. 3. NOMOGRAPH FOR OBTAINING Chi^2 FROM EXPECTED % AFFECTED AND OBSERVED–MINUS–EXPECTED (STEP 4).

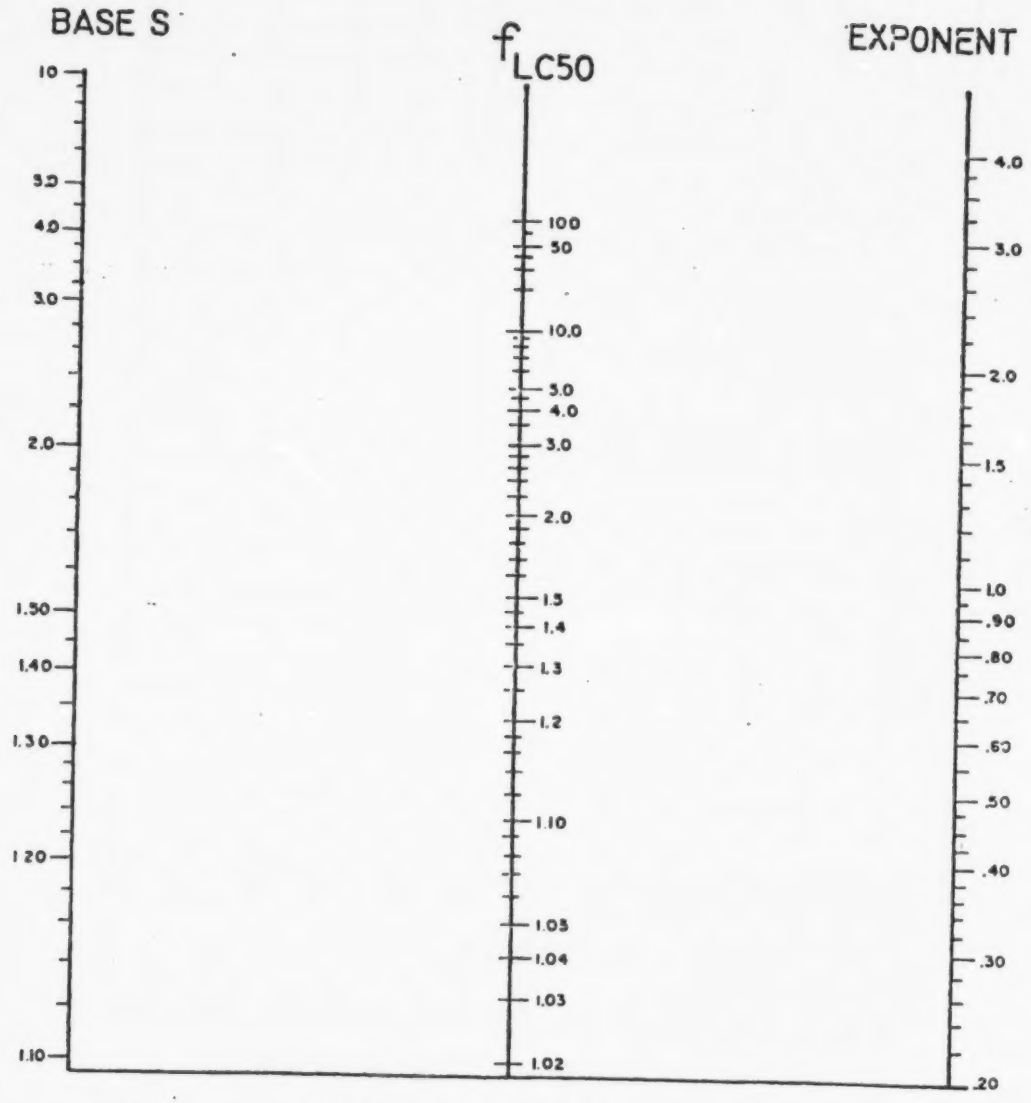

FIG. 4. NOMOGRAPH FOR RAISING BASE S TO A FRACTIONAL EXPONENT

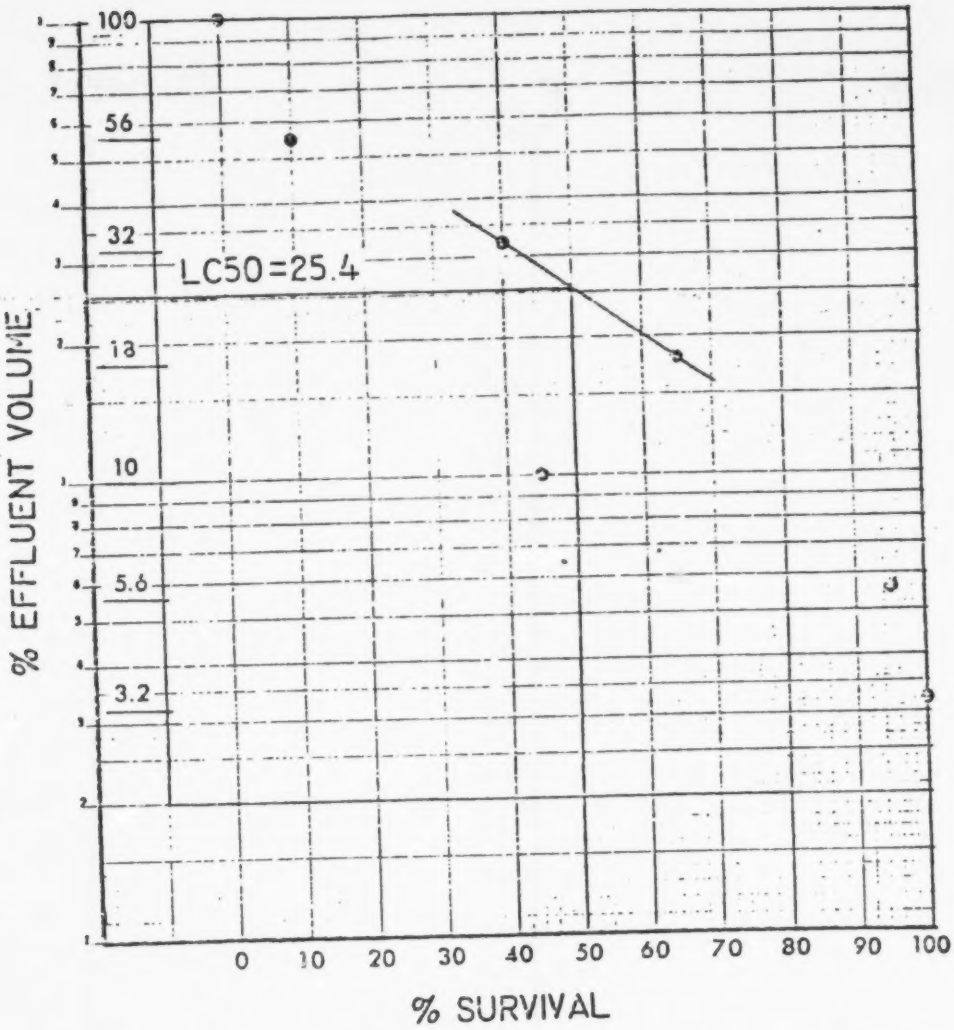

FIG. 5. PLOTTED DATA AND FITTED LINE FOR LOG-CONCENTRATION VERSUS % SURVIVAL METHOD

B. LOG-CONCENTRATION VERSUS PERCENT-SURVIVAL METHOD OF DETERMINING THE LC50

General Procedure

Step 1: Plot the percent effluent volumes and the corresponding percent survival on semi-logarithmic paper (Fig. 5).

Step 2: Locate the 2 highest points on the graph which are separated by the 50 percent survival line and connect them with a diagonal straight line. However, if one of the points is an aberrant value, the next lowest or highest percent-effluent volume is used.

Step 3: Read on the scale for percent-effluent volume, the value of the point where the diagonal line and the 50 percent survival line intersect. This value is the LC50 percent-effluent volume for the test. If by chance one of the effluent concentrations happens to have 50 percent survival, no graphing is necessary.

Example

Step 1: The percent-effluent volumes and the corresponding percent survival data from the Litchfield and Wilcoxon example are plotted in Fig. 5.

Step 2: The two highest points which are separated by the 50 percent survival line (65 percent and 40 percent) are located and connected with a diagonal diagonal straight line. The percent survival in the 10 percent-effluent volume was considered an aberrant value and, therefore, was omitted from the evaluation.

Step 3: An LC50 of 25.4 percent-effluent volume for the test was derived from the point where the diagonal line and the 50 percent survival line intersected in Fig.5.

SOURCE: Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms. EPA 600/4 78-012, revised July, 1978.

REPORTING TEST RESULTS

A report of the results of a test must include the following:

- The name of the test method, investigator and laboratory, and the date the test was conducted.
- A detailed description of the effluent, including its source, date and time of collection, composition, known physical and chemical properties, and variability.
- The source of the dilution water, the date and time of its collection, its chemical characteristics, and a description of any pretreatment.
- Detailed information about the test organisms, including scientific name, length and weight, age, life stage, source, history, observed diseases, treatments, and acclimation procedure used.
- A description of the test procedure and the test chambers, including the depth and volume of solution; the way the test was begun; the number of organisms per treatment; and the loading. For the flowthrough system, the water volume changes per 24 hours in each test chamber must be calculated and reported.
- The definition of the adverse effect (death, immobility, etc.) used in the test, and a summary of general observations on other effects or symptoms.
- The number and percentage of organisms in each test chamber (including the control chambers) that died or showed the "effect" used to measure the toxicity of the effluent.
- A 24-, 48-, 72-, and 96-hour LC_{50} or EC_{50} value for the test organisms, depending on the duration of exposure. If 100 percent effluent did not kill or affect more than 65 percent of the test organisms, report the percentage of the test organisms killed or affected by various concentrations of the effluent.
- The 95-percent confidence limits for the LC_{50} and EC_{50} values and the method used to calculate them.
- The methods used for the results of all chemical analyses.
- The average and range of the acclimation temperature and the test temperature.
- Any deviation from this method.
- Any other relevant information.

GENERAL BIOMONITORING COMPLIANCE SAMPLING INSPECTION DAILY ACTIVITIES

A. Day 1

1. Get power connected to mobile laboratory.
2. Level and stabilize laboratory.
3. Collect dilution water.
4. Begin acclimation.
5. Set up static rangefinding test
6. Make necessary entries in logbooks and fill in necessary forms.

B. Day 2

1. Check results of rangefinding test and make necessary logbook entries.
2. Assemble dilution board and delivery system.
3. Calibrate dilution board.
4. Activate diluter and begin filling test tanks.
5. Cease flow to acclimation tank.
5. If a composite sample is to be used for the static test, the compositer should be set up this day.
7. Collect dilution water.
8. Make all necessary logbook entries.

C. Day 3

1. Check all systems to ascertain that all have worked overnight.
2. Check temperatures to see if the acclimation temperature and test temperature are approximately the same.
3. Start the pump in the circulating water bath and turn the thermal equilizing unit on.

4. Collect the sample for the static test, whether it is grab or composite.

5. Set up static test tanks.

6. Perform temperature, dissolved oxygen, pH, and conductivity readings in all test containers.

7. Introduce the test organisms to both the static and flowthrough test containers.

8. Collect additional dilution water.

D. Days 4, 5, and 6

1. Check all systems to ascertain that all have worked overnight.

2. Record test organism mortality in all test containers and remove dead organisms where appropriate.

3. Perform length and weight measurements on dead fish (make necessary logbook entries).

4. Calibrate the appropriate meters and take meter readings.

5. Collect dilution water.

6. When scheduled, conduct a compliance biomonitoring evaluation inspection.

7. Make all necessary logbook entries.

E. Day 7

1. Check all systems to ascertain that all have worked overnight.

2. Record test organism mortality in all test containers and remove dead organisms where appropriate.

3. Calibrate the appropriate meters and take meter readings.

4. Recalibrate diluter board.

5. Make all necessary entries in logbooks.

6. Dismantle laboratory and secure equipment.

7. Inform permittee of your departure and sign out with gate security guard.

INSTRUCTIONS FOR COMPLETING THE ACUTE TOXICITY LABORATORY EVALUATION FORM

1. Laboratory or Industry - Enter the complete name of the laboratory or industry conducting the acute toxicity test.

1.a. Industry SIC Code - Enter this number and briefly describe the type of industry, raw materials used and estimated effluent composition if available.

2. Location - Enter the address of the laboratory or industry conducting the acute toxicity test.

2.a. NPDES Permit No. - Enter the corresponding number and other necessary permit identification such as date of issuance and expiration.

3. Date - Enter date of evaluation.

4. Investigator - Enter name and title of person conducting evaluation.

5. Company Representative - Enter name of person(s) interviewed and telephone number (if available).

6. Test Method - Enter brief narrative of the test being conducted and the reference where written instructions on the methodology appears (i.e. 96-hour static bioassay; or reference: EPA 660/3-75-009 April, 1975).

7.a. Dilution Water - Source - Enter the source of the dilution water; the date and time of its collection.

7.b. Diltuion Water: Chemical Analyses Performed - Enter specific

chemical tests performed on dilution water if any. Also enter chemical characteristics recorded by the analyst (average and/or range values).

7.c. Dilution Water: Pretreatment - Enter a description of any pretreatment of dilution water.

8.a. Effluent Water: Source - Enter the source of the effluent to be tested, the date and time of its collection.

8.b. Effluent Water: Variability - Enter a description of the physical or chemical variability of the effluent (i.e. constant flow of effluent from a lagoon with 14-days detention time or batch process releasing effluent having variable flow and chemistry directly into the receiving water).

8.c. Effluent Water: Sampling Technique - Enter a brief description of the method used to collect the sample(s) of effluent.

8.d. Effluent Water: Holding time and Conditions - Enter the amount of time and conditions under which the test effluent is held before being used in the toxicity study.

8.e. Effluent Water: Pretreatment - Enter a description of any pretreatment of the effluent.

8.f. Effluent Water: Chemical Analyses Performed - Enter specific chemical tests performed on effluent. Also enter chemical characteristics recorded by the analyst (average and/or range values).

9.a. Test Organism: Species - Enter the common and scientific name of the test organism.

9.b. Test Organism: Life State - Enter the age, life stage, as well as length and weight (if apporpriate) of the test organism.

9.c. Test Organism: Source - Enter the specific source of the test organism; the date and time of the collection (i.e. Brown Fish Hatchery, Central City, Iowa; collected 0800 hours on January 10, 1978).

9.d. Test Organism: Holding Facilities - Enter a brief description of the facility used to hold test organisms prior to the biomonitoring study (i.e. 500-gallon Minnow-Kool tank with flow-through dechlorinated tap water).

9.e. Test Organism: Acclimation Procedure - Enter a brief description of the procedure used to acclimate the test organism to laboratory conditions prior to biomonitoring tests.

9.f. Test Organism: Treatment - Enter any observed diseases and specific treatment rendered if any. State the number of treatments and dates.

10.a. Experimental Design: Equipment Cleaning Procedure - Enter a brief description of step-by-step pre-cleaning procedure for equipment (tanks, etc.) used in biomonitoring tests. List trade name and scientific name of cleaning compounds (if available).

10.b.(1) Experimental Design: Test Chambers: Construction Material - Enter the type of material used in constructing the test chambers.

10.b.(2) Experimental Design: Test Chambers: Dimensions - Enter the specific size of the test chambers (length, width, height).

10.b.(3) Experimental Design: Test Chambers: Volume - Enter the designated volume of the test chambers as well as the specific depth and volume of solution used during the biomonitoring test.

10. . Experimental Design: Test Chambers: Volumetric Exchange Rate - Enter the rate of exchange of test solution in flow through/continuous-flow test chambers.

10.c. Experimental Design: Test concentrations - Enter a list of solution concentrations in which test organisms were exposed.

10.d. Experimental Design: Number of organisms per concentration - Enter the number of test organisms exposed to each concentration of test solution.

10.e. Experimental Design: Loading Rate - Enter the weight of test organisms per liter of test solution (i.e. 5 grams/liter).

19.f. Experimental Design: Test Temperature - Average and Range - Enter the temperature (average and range) of the solution in which test organisms are exposed during the biomonitoring study.

.g Experimental Design: Chemical Parameters Monitored and Frequency - Enter the type of chemical tests performed and the frequency which each chemical test is performed during the biomonitoring study.

10.h. Experimental Design: Duration and Frequency of Test - Enter the time period of the biomonitoring test and the number of times the biomonitoring test is performed each year. (Record both as "performed" and "as required in NPDES permit").

10.i. Experimental Design: Definition of adverse effect - Define the endpoint of the biomonitoring test (i.e. death).

10.j. Experimental Design: Frequency of Observations - Enter the time intervals when test organisms were observed during the biomonitoring study (i.e. observed each 12-hour period).

.k. Experimental Design: Method of calculating EC50 or LC50 - Enter the name of the calculation procedure used and the reference citation.

10.1. Experimental Design: Special Conditions - Briefly describe test conditions not addressed elsewhere in this questionnaire (i.e. dead organisms not removed during the test; or test chambers aerated continuously with pure O2 during test). Attach a supplement sheet if needed.

11. Methods Used for All Chemical Analyses - Enter the reference cited for the chemical analyses performed during the biomonitoring study.

12. Other Relevant Information - Enter explanations of information provided elsewhere in the Acute Toxicity Laboratory Evaluation questionnaire or other pertinent information not presented in the audit questionnaire (i.e., quality assurance program, training and experience of analyst, adequacy of laboratory equipment and facilities, etc.).

ACUTE TOXICITY LABORATORY EVALUATION FORM

1. Laboratory or industry _____

   a. Industry or SIC Code _____

2. Location _____

   a. NPDES Permit No. _____

3. Date _____

4. Investigator _____

5. Company Representative _____

6. Test Method _____

7. Dilution Water

   a. Source _____

   Date: _____ Time _____

   b. Chemical Analyses Performed _____

c. Pretreatment _____

_____

_____

_____

_____

8. Effluent Water

a. Source _____

_____

b. Variability _____

_____

c. Sampling Technique _____

_____

d. Holding time and conditons _____

_____

e. Pretreatment _____

_____

f. Chemical analyses performed _____

_____

9. Test Organism

a. Species _____

_____

b. Life stage _____

_____

c. Source _____

_____

d. Holding facilities _____

_____

e. Acclimation Procedure_____

_____

f. Treatment (schematic or flow chart, if available)_____

_____

10. Experimental Design

a. Equipment Cleaning Procedure_____

_____

_____

b. Test Chambers

(1) Construction material_____

(2) Dimensions_____

(3) Volume_____

(4) Volumetric exchange rate_____

c. Test concentrations_____

_____

d. Number of organisms per concentration_____

_____

e. Loading rate_____

_____

f. Test temperature - average and range_____

_____

g. Chemical parameters monitored and frequency_____

_____

_____

h. Duration and frequency of test_____

_____

i. Definition of adverse effect_____

_____

j. Frequency of observations_____

_____

k. Method of calculating EC50 or LC50_____

_____

l. Special conditions_____

_____

11. Methods used for all chemical analyses_____

_____

_____

12. Record Keeping_____

_____

_____

_____

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_____

13. Other relevant information_____

_____

_____

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HANDOUT 16

Form Approved
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NPDES COMPLIANCE INSPECTION REPORT *(Coding Instructions on back of last page)*

TRANSACTION CODE	NPDES	YR MO DA	INSPEC-TOR	FAC TYPE	TIME
1 2 (5)	3 — 11	12 — 17	18	19	20	a.m. / p.m.

REMARKS (21)

ADDITIONAL (65 — 70) (64)

SECTION A - Permit Summary

NAME AND ADDRESS OF FACILITY *(Include County, State and ZIP code)*		EXPIRATION DATE
		ISSUANCE DATE
RESPONSIBLE OFFICIAL	TITLE	PHONE
FACILITY REPRESENTATIVE	TITLE	PHONE

SECTION B - Effluent Characteristics *(Additional sheets attached _____)*

PARAMETER/ OUTFALL		MINIMUM	AVERAGE	MAXIMUM	ADDITIONAL
	SAMPLE MEASUREMENT				
	PERMIT REQUIREMENT				
	SAMPLE MEASUREMENT				
	PERMIT REQUIREMENT				
	SAMPLE MEASUREMENT				
	PERMIT REQUIREMENT				
	SAMPLE MEASUREMENT				
	PERMIT REQUIREMENT				
	SAMPLE MEASUREMENT				
	PERMIT REQUIREMENT				

SECTION C - Facility Evaluation *(S = Satisfactory, U = Unsatisfactory, N/A = Not applicable)*

	EFFLUENT WITHIN PERMIT REQUIREMENTS		OPERATION AND MAINTENANCE		SAMPLING PROCEDURES
	RECORDS AND REPORTS		COMPLIANCE SCHEDULE		LABORATORY PRACTICES
	PERMIT VERIFICATION		FLOW MEASUREMENTS		OTHER:

SECTION D - Comments

SECTION E - Inspection/Review

SIGNATURES	AGENCY	DATE
INSPECTED BY		
INSPECTED BY		
REVIEWED BY		

ENFORCEMENT DIVISION USE ONLY

COMPLIANCE STATUS

☐ COMPLIANCE

☐ NONCOMPLIANCE

HANDOUT 16

Form Approved
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Sections F thru L: Complete on all inspections, as appropriate. N/A = Not Applicable

PERMIT NO.

SECTION F - Facility and Permit Background

ADDRESS OF PERMITTEE IF DIFFERENT FROM FACILITY *(Including City, County and ZIP code)*

DATE OF LAST PREVIOUS INVESTIGATION BY EPA/STATE

FINDINGS

SECTION G - Records and Reports

RECORDS AND REPORTS MAINTAINED AS REQUIRED BY PERMIT. ☐ YES ☐ NO ☐ N/A *(Further explanation attached _____)*
DETAILS:

(a) ADEQUATE RECORDS MAINTAINED OF:			
(i) SAMPLING DATE, TIME, EXACT LOCATION	☐ YES	☐ NO	☐ N/A
(ii) ANALYSES DATES, TIMES	☐ YES	☐ NO	☐ N/A
(iii) INDIVIDUAL PERFORMING ANALYSIS	☐ YES	☐ NO	☐ N/A
(iv) ANALYTICAL METHODS/TECHNIQUES USED	☐ YES	☐ NO	☐ N/A
(v) ANALYTICAL RESULTS *(e.g., consistent with self-monitoring report data)*	☐ YES	☐ NO	☐ N/A
(b) MONITORING RECORDS *(e.g., flow, pH, D.O., etc.)* MAINTAINED FOR A MINIMUM OF THREE YEARS INCLUDING ALL ORIGINAL STRIP CHART RECORDINGS *(e.g. continuous monitoring instrumentation, calibration and maintenance records).*	☐ YES	☐ NO	☐ N/A
(c) LAB EQUIPMENT CALIBRATION AND MAINTENANCE RECORDS KEPT.	☐ YES	☐ NO	☐ N/A
(d) FACILITY OPERATING RECORDS KEPT INCLUDING OPERATING LOGS FOR EACH TREATMENT UNIT.	☐ YES	☐ NO	☐ N/A
(e) QUALITY ASSURANCE RECORDS KEPT.	☐ YES	☐ NO	☐ N/A
(f) RECORDS MAINTAINED OF MAJOR CONTRIBUTING INDUSTRIES *(and their compliance status)* USING PUBLICLY OWNED TREATMENT WORKS.	☐ YES	☐ NO	☐ N/A

SECTION H - Permit Verification

INSPECTION OBSERVATIONS VERIFY THE PERMIT. ☐ YES ☐ NO ☐ N/A *(Further explanation attached _____)*
DETAILS:

(a) CORRECT NAME AND MAILING ADDRESS OF PERMITTEE.	☐ YES	☐ NO	☐ N/A
(b) FACILITY IS AS DESCRIBED IN PERMIT.	☐ YES	☐ NO	☐ N/A
(c) PRINCIPAL PRODUCT(S) AND PRODUCTION RATES CONFORM WITH THOSE SET FORTH IN PERMIT APPLICATION.	☐ YES	☐ NO	☐ N/A
(d) TREATMENT PROCESSES ARE AS DESCRIBED IN PERMIT APPLICATION.	☐ YES	☐ NO	☐ N/A
(e) NOTIFICATION GIVEN TO EPA/STATE OF NEW, DIFFERENT OR INCREASED DISCHARGES.	☐ YES	☐ NO	☐ N/A
(f) ACCURATE RECORDS OF RAW WATER VOLUME MAINTAINED.	☐ YES	☐ NO	☐ N/A
(g) NUMBER AND LOCATION OF DISCHARGE POINTS ARE AS DESCRIBED IN PERMIT.	☐ YES	☐ NO	☐ N/A
(h) CORRECT NAME AND LOCATION OF RECEIVING WATERS.	☐ YES	☐ NO	☐ N/A
(i) ALL DISCHARGES ARE PERMITTED.	☐ YES	☐ NO	☐ N/A

SECTION I - Operation and Maintenance

TREATMENT FACILITY PROPERLY OPERATED AND MAINTAINED. ☐ YES ☐ NO ☐ N/A *(Further explanation attached _____)*
DETAILS:

(a) STANDBY POWER OR OTHER EQUIVALENT PROVISIONS PROVIDED.	☐ YES	☐ NO	☐ N/A
(b) ADEQUATE ALARM SYSTEM FOR POWER OR EQUIPMENT FAILURES AVAILABLE.	☐ YES	☐ NO	☐ N/A
(c) REPORTS ON ALTERNATE SOURCE OF POWER SENT TO EPA/STATE AS REQUIRED BY PERMIT.	☐ YES	☐ NO	☐ N/A
(d) SLUDGES AND SOLIDS ADEQUATELY DISPOSED.	☐ YES	☐ NO	☐ N/A
(e) ALL TREATMENT UNITS IN SERVICE.	☐ YES	☐ NO	☐ N/A
(f) CONSULTING ENGINEER RETAINED OR AVAILABLE FOR CONSULTATION ON OPERATION AND MAINTENANCE PROBLEMS.	☐ YES	☐ NO	☐ N/A
(g) QUALIFIED OPERATING STAFF PROVIDED.	☐ YES	☐ NO	☐ N/A
(h) ESTABLISHED PROCEDURES AVAILABLE FOR TRAINING NEW OPERATORS.	☐ YES	☐ NO	☐ N/A
(i) FILES MAINTAINED ON SPARE PARTS INVENTORY, MAJOR EQUIPMENT SPECIFICATIONS, AND PARTS AND EQUIPMENT SUPPLIERS.	☐ YES	☐ NO	☐ N/A
(j) INSTRUCTIONS FILES KEPT FOR OPERATION AND MAINTENANCE OF EACH ITEM OF MAJOR EQUIPMENT.	☐ YES	☐ NO	☐ N/A
(k) OPERATION AND MAINTENANCE MANUAL MAINTAINED.	☐ YES	☐ NO	☐ N/A
(l) SPCC PLAN AVAILABLE.	☐ YES	☐ NO	☐ N/A
(m) REGULATORY AGENCY NOTIFIED OF BY PASSING. *(Dates _____ _____)*	☐ YES	☐ NO	☐ N/A
(n) ANY BY-PASSING SINCE LAST INSPECTION.	☐ YES	☐ NO	☐ N/A
(o) ANY HYDRAULIC AND/OR ORGANIC OVERLOADS EXPERIENCED.	☐ YES	☐ NO	☐ N/A

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PERMIT NO.

SECTION J - Compliance Schedules

PERMITTEE IS MEETING COMPLIANCE SCHEDULE. ☐ YES ☐ NO ☐ N/A *(Further explanation attached ____)*

CHECK APPROPRIATE PHASE(S)

- ☐ (a) THE PERMITTEE HAS OBTAINED THE NECESSARY APPROVALS FROM THE APPROPRIATE AUTHORITIES TO BEGIN CONSTRUCTION.
- ☐ (b) PROPER ARRANGEMENT HAS BEEN MADE FOR FINANCING *(mortgage commitments, grants, etc.)*.
- ☐ (c) CONTRACTS FOR ENGINEERING SERVICES HAVE BEEN EXECUTED.
- ☐ (d) DESIGN PLANS AND SPECIFICATIONS HAVE BEEN COMPLETED.
- ☐ (e) CONSTRUCTION HAS COMMENCED.
- ☐ (f) CONSTRUCTION AND/OR EQUIPMENT ACQUISITION IS ON SCHEDULE.
- ☐ (g) CONSTRUCTION HAS BEEN COMPLETED.
- ☐ (h) START-UP HAS COMMENCED.
- ☐ (i) THE PERMITTEE HAS REQUESTED AN EXTENSION OF TIME.

SECTION K - Self-Monitoring Program

Part 1 – Flow measurement *(Further explanation attached ____)*

PERMITTEE FLOW MEASUREMENT MEETS THE REQUIREMENTS AND INTENT OF THE PERMIT. DETAILS:	☐ YES	☐ NO	☐ N/A
(a) PRIMARY MEASURING DEVICE PROPERLY INSTALLED.	☐ YES	☐ NO	☐ N/A
TYPE OF DEVICE: ☐ WEIR ☐ PARSHALL FLUME ☐ MAGMETER ☐ VENTURI METER ☐ OTHER *(Specify ____)*			
(b) CALIBRATION FREQUENCY ADEQUATE. *(Date of last calibration ____)*	☐ YES	☐ NO	☐ N/A
(c) PRIMARY FLOW MEASURING DEVICE PROPERLY OPERATED AND MAINTAINED.	☐ YES	☐ NO	☐ N/A
(d) SECONDARY INSTRUMENTS *(totalizers, recorders, etc.)* PROPERLY OPERATED AND MAINTAINED.	☐ YES	☐ NO	☐ N/A
(e) FLOW MEASUREMENT EQUIPMENT ADEQUATE TO HANDLE EXPECTED RANGES OF FLOW RATES.	☐ YES	☐ NO	☐ N/A

Part 2 – Sampling *(Further explanation attached ____)*

PERMITTEE SAMPLING MEETS THE REQUIREMENTS AND INTENT OF THE PERMIT. DETAILS:	☐ YES	☐ NO	☐ N/A
(a) LOCATIONS ADEQUATE FOR REPRESENTATIVE SAMPLES.	☐ YES	☐ NO	☐ N/A
(b) PARAMETERS AND SAMPLING FREQUENCY AGREE WITH PERMIT.	☐ YES	☐ NO	☐ N/A
(c) PERMITTEE IS USING METHOD OF SAMPLE COLLECTION REQUIRED BY PERMIT. IF NO ☐ GRAB ☐ MANUAL COMPOSITE ☐ AUTOMATIC COMPOSITE FREQUENCY ____	☐ YES	☐ NO	☐ N/A
(d) SAMPLE COLLECTION PROCEDURES ARE ADEQUATE.	☐ YES	☐ NO	☐ N/A
(i) SAMPLES REFRIGERATED DURING COMPOSITING	☐ YES	☐ NO	☐ N/A
(ii) PROPER PRESERVATION TECHNIQUES USED	☐ YES	☐ NO	☐ N/A
(iii) FLOW PROPORTIONED SAMPLES OBTAINED WHERE REQUIRED BY PERMIT	☐ YES	☐ NO	☐ N/A
(iv) SAMPLE HOLDING TIMES PRIOR TO ANALYSES IN CONFORMANCE WITH 40 CFR 136.3	☐ YES	☐ NO	☐ N/A
(e) MONITORING AND ANALYSES BEING PERFORMED MORE FREQUENTLY THAN REQUIRED BY PERMIT.	☐ YES	☐ NO	☐ N/A
(f) IF (e) IS YES, RESULTS ARE REPORTED IN PERMITTEE'S SELF-MONITORING REPORT.	☐ YES	☐ NO	☐ N/A

Part 3 – Laboratory *(Further explanation attached ____)*

PERMITTEE LABORATORY PROCEDURES MEET THE REQUIREMENTS AND INTENT OF THE PERMIT. DETAILS:	☐ YES	☐ NO	☐ N/A
(a) EPA APPROVED ANALYTICAL TESTING PROCEDURES USED. *(40 CFR 136.3)*	☐ YES	☐ NO	☐ N/A
(b) IF ALTERNATE ANALYTICAL PROCEDURES ARE USED, PROPER APPROVAL HAS BEEN OBTAINED.	☐ YES	☐ NO	☐ N/A
(c) PARAMETERS OTHER THAN THOSE REQUIRED BY THE PERMIT ARE ANALYZED.	☐ YES	☐ NO	☐ N/A
(d) SATISFACTORY CALIBRATION AND MAINTENANCE OF INSTRUMENTS AND EQUIPMENT.	☐ YES	☐ NO	☐ N/A
(e) QUALITY CONTROL PROCEDURES USED.	☐ YES	☐ NO	☐ N/A
(f) DUPLICATE SAMPLES ARE ANALYZED. ____ % OF TIME.	☐ YES	☐ NO	☐ N/A
(g) SPIKED SAMPLES ARE USED. ____ % OF TIME.	☐ YES	☐ NO	☐ N/A
(h) COMMERCIAL LABORATORY USED.	☐ YES	☐ NO	☐ N/A
(i) COMMERCIAL LABORATORY STATE CERTIFIED.	☐ YES	☐ NO	☐ N/A

LAB NAME ____

LAB ADDRESS ____

HANDOUT 16

Form Approved
OMB No. 158 - R0073

PERMIT NO.

SECTION L - Effluent/Receiving Water Observations *(Further explanation attached _____)*

OUTFALL NO.	OIL SHEEN	GREASE	TURBIDITY	VISIBLE FOAM	VISIBLE FLOAT SOL	COLOR	OTHER

(Sections M and N: Complete as appropriate for sampling inspections)

SECTION M - Sampling Inspection Procedures and Observations *(Further explanation attached _____)*

- ☐ GRAB SAMPLES OBTAINED
- ☐ COMPOSITE OBTAINED
- ☐ FLOW PROPORTIONED SAMPLE
- ☐ AUTOMATIC SAMPLER USED
- ☐ SAMPLE SPLIT WITH PERMITTEE
- ☐ CHAIN OF CUSTODY EMPLOYED
- ☐ SAMPLE OBTAINED FROM FACILITY SAMPLING DEVICE

COMPOSITING FREQUENCY _____ PRESERVATION _____

SAMPLE REFRIGERATED DURING COMPOSITING: ☐ YES ☐ NO

SAMPLE REPRESENTATIVE OF VOLUME AND NATURE OF DISCHARGE _____

SECTION N - Analytical Results *(Attach report if necessary)*

INSTRUCTIONS FOR COMPLETING THE NPDES COMPLIANCE INSPECTION REPORT (EPA Form 3560-3)

Overview

The intent of the NPDES Compliance Inspection Report form is to provide standard, reviewable information about an inspection to Enforcement. All inspections will be conducted and all reports will be completed as if they may lead to enforcement action. The form defines the minimum amount of information that Enforcement should receive. Regional and State inspectors may elect to include additional information, as the circumstances warrant.

Both Compliance Evaluation Inspections (CEIs) and Compliance Sampling Inspections (CSIs) of municipal and non-municipal facilities will be conducted using the same type of Compliance Inspection Report form (EPA Form 3560-3). Using the same form and format will minimize the reporting burden on inspectors and permittees because identical elements of compliance (e.g., permittee records and self-monitoring program, etc.) are examined in both CEIs and CSIs. Although the form may be used for either inspection, a completed form will be credited in only one category of the Formal Program Reporting System (FPRS). A completed form contains all the information appropriate to the accomplished inspection. A completed form is, by definition, also what will be accepted by the Enforcement Director of the agency responsible for enforcing the permit. Procedures for the distribution of the completed form should be planned with the Enforcement Director. Users should note that the top of page 1 serves as a coding sheet for entries into the Water Enforcement National Data Base (WENDB).

The Compliance Inspection Report consists of two major parts. The first part, sections A-L, is completed for all inspections, as appropriate. The second part, sections M-N, is completed only for CSIs. For the checklists, sections G through K, each lead statement will summarize deficiencies covered in the section. Each item in the checklist (except Section I items (n) & (o)) is written so that a "yes" answer is positive, indicating some degree of permit compliance. If there are no problems in a section, all the answers will be yes (with the exceptions noted above).

Throughout the form, numerous opportunities exist to attach additional explanations. These explanations should be attached only when necessary. Although a narrative is not appropriate when a simple yes, no, or N/A will do, inspectors must adequately document their observations. However, lengthy narratives will defeat the purpose of the checklists. Nonetheless, if further explanation is deemed necessary, it should be attached and noted on the form.

A brief review of each section follows. For further explanation of Compliance Inspections, consult the appropriate manuals. (NPDES Compliance Evaluation Inspection Manual - U.S. EPA, Office of Water Enforcement and NPDES Compliance Sampling Manual - U.S. EPA, Office of Water Enforcement).

Keypunch Summary

This lead information is used to identify the facility and the inspection date, type and agency. The data can be keypunched on one card and entered directly into the Water Enforcement National Data Base (WENDB). Entries in WENDB will assist tracking of inspection results and will be used for reporting in FPRS. To be part of the WENDB, the data should be reported as follows:

Column 1	Transaction Code - Use N, C, or D for New, Change or Delete. All inspections will be new unless there is an error in the data keypunched into WENDB.
Column 2	Card Code - always 5 for this card.
Columns 3-11	NPDES - The NPDES permit number. (The State permit number may be accommodated in the remarks or additional spaces).
Columns 12-17	Inspection Date - entered in the year/month/day format (e.g. 77/06/30 = June 30, 1977).
Column 18	Inspection Type - An inspection will fall into one of two possible categories: 'C' for Compliance Evaluation or 'S' for Compliance Sampling.

Column 19 — Inspector Code - An inspection may be performed by the Region, State or NEIC (U.S. EPA National Enforcement Investigations Center). It may also be the result of a joint effort. (Credit in FPRS for a joint inspection is given to the lead agency.) Acceptable codes for WENDB are:

- R - EPA Regional inspections
- S - State inspections
- J - Joint EPA and State inspections - EPA lead
- T - Joint EPA and State inspections - State lead
- N - NEIC inspections

Column 20 — Facility Type - This code describes the type of facility that was inspected. Acceptable codes are:

- 1 - Municipal - Publicly-Owned Treatment Works (POTWs) with 1972 Standard Industrial Classification (SIC) 4952
- 2 - Industrial - Other than Municipal, Agricultural, and Federal facilities.
- 3 - Agricultural - Those facilities classified with 1972 SIC 0111-0971.
- 4 - Federal - Those facilities identified as Federal by EPA Regional office.

Columns 21-70 — Remarks - This remarks field provides the inspector with a vehicle to store descriptive information about the inspection. There is no set format within this 50-position field. Individual Regions or States may choose to set aside portions of this field for their own specific needs.

The "Time" and "Additional" boxes can also describe the inspection, but will not be keypunched. Supplementary information that the performing agency or Region needs may be entered in the Additional box, e.g., STORET numbers, basin codes, etc.

Section A - Permit Summary

This section provides the summary information required to further identify the inspected facility. Most of the elements are self explanatory; however, the last two lines may require explanation. "Responsible Official" is the individual required to sign the Discharge Monitoring Report or is responsible for wastewater management at the facility. "Facility Representative" is the individual who acted as a contact during the inspection.

Section B - Effluent Characteristics

Effluent Characteristics contains a summary of those parameters (e.g. BOD, pH, flow) that are regulated by the permit and any other parameters that are measured but not regulated by the permit. If more than one outfall is inspected, the parameter and outfall should be indicated and additional sheets attached as required. If the inspection will not include samples, it may be advisable, but is not required, to substitute the data from the latest Discharge Monitoring Report in the "Sample Measurement" row before performing the inspection. However, if self-monitoring data are entered in the spots for sampling data, they should be clearly identified as such to avoid confusing the reviewer. The column marked "Additional" is for the performing agency's or Region's own requirements, e.g., design data, comments or explanations of the measurements.

Section C - Facility Evaluation

The Facility Evaluation provides a summary evaluation of the inspection results. The evaluations made in this section should be documented and supported by notation in the appropriate checklist portions of the form and by any additional comments as required.

Section D - Comments

Little space is allowed for comments here. Rather than fragmenting the narrative detailing comments and possible recommendations, the form allows detailed comments in an

attachment, on the back of the form, or in Section N. The Section D comments should be used to flag lengthy comments (e.g. "Recommendations on p.4") or used for those inspections which only merit abbreviated comments. Procedures for making recommendations and comments should be worked out with the Enforcement Director of the organization responsible for the permit. All comments or recommendations that are made should be documented and supported by the checklist portions of the form.

Section E - Inspection Review

This section provides the inspector's and reviewer's names and agencies. Compliance status should be determined only by the Enforcement personnel.

Section F - Facility and Permit Background

If the permittee's address is different from that of the facility, it should be so indicated. If the facility was inspected previously, the date and findings summary should be noted before performing the current inspection.

Section G - Records and Reports

This portion of the form documents that the records and reports maintained by the permittee are in compliance with permit requirements. As mentioned earlier, if the checklist does not adequately represent the situation, further explanation should be attached and so indicated.

Section H - Permit Verification

Each inspection should identify discrepancies, if any, between the issued permit and actual conditions. Again, if further explanation is necessary, it should be provided and so indicated.

Section I - Operation and Maintenance

Each inspection of an operating facility should evaluate its operation and maintenance. Operating facilities include those on final limits and those in the process of being upgraded.

Section J - Compliance Schedule

The compliance schedule progress should be evaluated when the permittee is on a compliance schedule. Any grant-related inspections of facilities should be coordinated with Regional Construction Grants personnel. The current phase of compliance schedule status should be marked on the form.

Section K - Self-Monitoring Program

The permittee's flow measurement, sampling, and laboratory procedures should be checked, as appropriate, on all inspections. If deficiencies are noted, additional pertinent information should be provided, if necessary. For example, if the laboratory is not calibrating or maintaining the equipment satisfactorily, the calibration or maintenance intervals should be noted. If parameters other than those required by the permit are analyzed, the parameters and analytical methods should be noted. If the permittee laboratory, flow-measurement, or sampling procedures are not inspected, an explanation should be provided (e.g., contract lab off the premises).

Section L - Effluent/Receiving Water Observations

Visual observations made during the inspection should be noted, as applicable, for each outfall. The inspector's observations are subjective and qualitative, but serve to focus attention on potential treatment problems. Discharge of floating solids or visible foam in other than trace amounts is prohibited by the permit. Thus, observations of greater than trace amounts represent permit violations and indicate poor treatment.

Section M - Sampling Inspection Procedures and Observations

The performing agency's or Region's sampling procedures should be noted for each sampling inspection. Details documenting the procedures should be provided (e.g., the composite time interval).

Section N - Analytical Results

If the analytical results or laboratory report from a sampling inspection provides more information than can be inserted in Section C, the additional information should be noted in this part or attached to the report form. This section also offers more space for comments or additional materials (e.g. flow diagrams) as the situation merits.